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Original Article

Impact of Herbal Preparations on Outcomes of Highly Active Antiretroviral Therapy: A One-Year Prospective Cohort Study*

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ABSTRACT **Objective**: To investigate the impacts of two herbal preparations for human immunodeficiency virus/aquired immune deficiency syndrome (HIV/AIDS) patients, Shenling Fuzheng Capsule (参灵扶正胶囊, SLFZC) and Qingdu Capsule (清毒胶囊, QDC), on the efficacy of highly active antiretroviral therapy (HAART). **Methods**: HIV/AIDS patients met the criteria were all enrolled in a 1-year cohort study, in which patients receiving HAART alone were designated as Group A, those receiving HAART in combination with SLFZC were designated as Group B, and those receiving HAART in combination with QDC were designated as Group C, 100 cases in each group. The dose of SLFZC was 1.48 g (4 capsules), 3 times daily, and QDC 1.56 g (4 capsules), 3 times daily. T cell subsets, HIV RNA and HIV-1 drug resistance were detected at enrollment and 1 year after treatment. Patients were followed up every 3 months, during which side-effects and other clinical data were recorded. **Results**: After 1-year treatment, the median increment in CD_4 counts was 165.0, 178.0 and 145.0 cells/μL for Group A, B and C, respectively. HIV RNA was undetectable in 94% of patients in Group A, 96% in Group B and 92% in Group C. There were no differences regarding the increment in CD_4 counts, HIV RNA and frequency of HIV-1 drug resistance mutations. Two of the 14 suspected side-effect symptoms, i.e. fatigue and dizziness, were lower in Groups B and C than in Group A (P<0.05, respectively). **Conclusion**: SLFZC and QDC do not have a negative impact on immunological and virological response to HAART; however, these preparations are not as potent in reducing HAART-associated side-effects as anticipated.

KEYWORDS human immunodeficiency syndrome, aquired immune deficiency syndrome, Chinese medicine, Shenling Fuzheng Capsule, Qingdu Capsule

Complementary and alternative medicine (CAM), including Chinese medicine (CM), has often been used by human immunodeficiency virus/aquired immune deficiency syndrome (HIV/AIDS) patients to boost or maintain immunity, treat symptoms, or alleviate side-effects of antiretroviral drugs.[1,2] However, it is argued that there are insufficient evidences to support the use of herbal medicines in these patients.[3,4] In addition, concerns have arisen that drug interactions may cause uncertain risks, including increased side-effects and decreased antiviral potency.[5,6]

Shenling Fuzheng Capsule (参灵扶正胶囊, SLFZC) and Qingdu Capsule (清毒胶囊, QDC) are two Chinese herbal preparations made by Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine and approved by local authorities to be used as complementary treatments for HIV/AIDS patients since 2011. To date, these two preparations have been used in 16 local hospitals. Our previous study showed that the use of SLFZC or QDC without highly active antiretroviral therapy (HAART) can postpone the time of HIV infection turning into AIDS, reduce the morbidity of AIDS, and improve the quality of life of patients.[7] However, unlike China State Food and Drug Administration-approved medicine, hospital-made CM preparations, including SLFC and QDC, do not undergo highly rigorous assessments before their clinical uses. In a retrospective database analysis, SLFZC and QDC were found to relieve

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some symptoms and mitigate side-effects associated with HAART, such as fatigue and headache.[8] Another study reported that SLFZC can alleviate HAART-associated diarrhea.[9] However, some clinicians attributed these effects to decreased blood concentrations of antiretroviral drugs resulting from drug reactions with herbal components in the two preparations. The aim of this study was to evaluate the impact of SLFZC and QDC on the effectiveness and side-effects of common HAART regimens, as well as HAART-related HIV-1 drug resistance mutations.

METHODS

Inclusion and Exclusion Criteria

Inclusion criteria include: HIV-1 infected individuals with a diagnosis of qi deficiency or damp-heat based on CM theory, naive to antiretroviral drugs, SLFZC and QDC at the time of enrollment, an age range of 18–65 years old, written informed consent has been obtained.

Exclusion criteria include: presence of vital organ dysfunction, with malignant tumors, a history of severe allergy to drugs, likely to be unavailable for follow-up.

Study Subjects and Intervention

The study subjects were recruited during February 2015 to June 2016 from Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, Red Cross Hospital of Yulin, People's Hospital of Youjiang District of Baise City, Qinzhou Hospital of Chinese Medicine and Hengxian Hospital of Chinese Medicine.

Patients met the above criteria were all enrolled in a 1-year cohort study, instead of being sampled from the target populations. There were 73 males and 27 females in Group A, 61 males and 39 females in Group B, 72 males and 28 females in Group C. The age was 48.3±15.4, 48.5±14.3 and 45.2±16.1 years in Group A, B and C, respectively. There were no statistically significant differences among the groups with respect to sex (χ^2=4.121, *P*=0.127) and age (*F*=1.452, *P*=0.236).

These patients were about to receive HAART alone (Group A), HAART combined with SLFZC (Group B), or HAART combined with QDC (Group C) at enrollment. Group A had 50 subjects with qi deficiency and 50 with damp-heat syndrome, Group B had 100 subjects with qi deficiency, and Group C had 100 subjects with damp-heat syndrome. They were matched for HAART regimens, and the number of patients receiving 3 different HAART combinations, zidovudine/lamivudine/efavirenz (AZT/3TC/EFV), tenofovir/lamivudine/efavirenz (TDF/3TC/EFV) and zidovudine/lamivudine/nevirapine (AZT/3TC/NVP) was 43, 18 and 39 respectively in each group. HAART was given according to the Guideline of Diagnosis and Treatment for AIDS by AIDS Group, Society of Infectious Diseases, Chinese Medical Association.

SLFZC is used as a tonic for qi weakness, consisting of rude extracts from *Codonopsis pilosula*, *Astragalus membranaceus*, *Bletilla striata*, *Ganoderma lucidum*, *Gynostemma pentaphyllum* and *Polyrhachis vicina*. QDC is used to clear damp-heat, containing rude extracts from *Astragalus membranaceus*, *Atractylodes lancea*, *Scutellaria baicalensis*, *Andrographis paniculata*, *Gynostemma pentaphyllum*, *Wolfiporia extens*, *Coix lacryma-jobi* var. ma-yuen, *Amomum villosum*, *Ganoderma lucidum* and *Polyrhachis vicina*. The dose of SLFZC was 1.48 g (4 capsules), 3 times daily, and QDC 1.56 g (4 capsules), 3 times daily.

After treatment, patients were followed up every 3 months, during which side-effects and other clinical data were recorded.

Sample Detection

Patients' blood samples were collected at the beginning and the end of the observation period for detection of T cell subsets and HIV RNA, as well as HIV-1 drug resistance mutations when HIV RNA ⩾1000 copies/mL. CD_4 and CD_8 cells were enumerated using a FACSCalibur flow cytometer (BD Biosciences). HIV RNA was measured using Roche COBAS® Amplicor™ HIV-1 Monitor® Test (Roche Diagnostics). To detect HIV-1 drug resistance mutations, RNA was isolated from plasma by the High Pure Viral RNA Kit (Roche Diagnostics), and HIV-1 pol gene was amplified between positions 2068 to 5221 in HXB2 reference strain by reverse transcriptase-polymerase chain reaction (RT-PCR). The amplified products were sequenced, and sequences obtained were submitted to the HIV Drug Resistance Database at Stanford University (http://hivdb.stanford.edu) to determine whether the sample contained HIV-1 drug resistance mutations.

Statistical Analysis

Statistic analysis was performed using SPSS 18.0

software (SPSS Inc., Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) when normal distribution and equal variance were met, or median (P25, P75) if data did not meet normal distribution and/or equal variance, comparison of which was subjected to one-way ANOVA with Student-Newman-Keuls post hoc tests or Kruskal-Wallis H test followed by Nemenyi's test. Count data were expressed as *n* (%) and were analyzed using Chi-square test or Fisher's exact test. *P* values less than 0.05 were considered statistical significant.

RESULTS

Clinical Characteristics

The clinical characteristics of the participants were shown in Table 1. Before the initiation of treatments, all participants had HIV RNA levels of more than 1000 copies/mL. There were no statistical differences in CD_4 and CD_8 cell counts, $\log_{10}$ HIV RNA, as well as the frequency of HIV-1 drug resistance mutations across groups ($P>0.05$). All patients completed the study and did not change medications during the observation period.

Clinical Outcomes during Follow-Up

The clinical outcomes after 1-year treatment are shown in Table 2. At the end of the observation period, the median increment in CD_4 cell counts in Groups A, B and C was 165.0, 178.0 and 145.0 cells/μL, respectively. Most patients (94.0% in Group A, 96.0% in Group B and 92.0% in Group C) had undetectable HIV RNA (<50 copies/mL). Only 3 patients, 1 in Group A and 2 in Group C, had HIV RNA higher than 1000 copies/mL, each harboring nucleoside reverse

Table 1. Clinical Characteristics of Study Participants

Item	Group A	Group B	Group C	χ^2/F	*P*
CD_4 [cells/μL, median (P25, P75)]	287.0 (188.0, 392.5)	290.0 (159.0, 427.0)	274.5 (155.8, 402.3)	1.256	0.534[c]
CD_8 [cells/μL, median (P25, P75)]	838.0 (647.5, 1220.0)	832.0 (565.0, 1191.0)	808.0 (640.8, 1170.8)	0.576	0.750[c]
HIV RNA ($\log_{10}$ copies/mL, $\bar{x} \pm s$)	4.2 ± 0.9	4.4 ± 0.9	4.4 ± 0.9	1.046	0.353[b]
Route of transmission [*n* (%)]					
Sexual exposure	94 (94.0)	98 (98.0)	97 (97.0)	2.454	0.293[a]
Injecting drug use	6 (6.0)	2 (2.0)	3 (3.0)		
Resistance mutation [*n* (%)]	9 (9.0)	13 (13.0)	10 (10.0)	0.910	0.635[a]
HAART regimen [*n* (%)]					
Zidovudine/lamivudine/efavirenz	43 (43.0)	43 (43.0)	43 (43.0)	–	–
Tenofovir/lamivudine/efavirenz	18 (18.0)	18 (18.0)	18 (18.0)		
Zidovudine/lamivudine/nevirapine	39 (39.0)	39 (39.0)	39 (39.0)		

Notes: *P*-value was calculated using [a]Chi-square test, [b]one-way ANOVA or [c]Kruskal-Wallis H test.

Table 2. Comparison of Clinic Outcomes and HIV-1 Drug Resistance among Groups

Clinical outcome	Group A	Group B	Group C	χ^2	*P*
CD_4 [cells/μL; median (P25, P75)]	362.5 (255.0, 468.0)	395.0 (303.5, 470.0)	375.0 (289.0, 458.5)	1.265	0.531[a]
Increment in CD_4 counts [cells/μL; median (P25, P75)]	165.0 (39.5, 248.5)	178.0 (52.5, 257.5)	145.0 (57.0, 201.0)	1.860	0.395[a]
CD8 [cells/μL; media (P25, P75)]	990.0 (788.5, 1198.8)	736.50 (575.3, 1305.3)	639.8 (901.5, 1190.0)	5.195	0.074[a]
HIV RNA [*n* (%)]					
<50 copies/mL	94 (94.0)	96 (96.0)	92 (92.0)	–	0.660[b]
<1000 copies/mL	5 (5.0)	4 (4.0)	6 (6.0)		
≥1000 copies/mL	1 (1.0)	0 (0.0)	2 (2.0)		
HIV-1 resistance mutation	1 (1.0)	0 (0.0)	2 (2.0)	–	0.776[b]
Drug resistance mutations					
NRTI-related mutations	L74I, V75M, T69Ins	–	M184V[1,2], M41L[1], L210W[1], T215Y[1],	–	–
NNRTI-related mutations	K103S, V106M, Y181V, Y188H, G190E, P236L	–	Y181V[1], K103N[2]	–	–

Notes: *P*-value was calculated using Kruskal-Wallis H test (a) or Fisher's exact Test (b). The superscript number (1 or 2) indicates the mutation was found in either of the 2 patients.

transcriptase inhibitor (NRTI)-associated mutations and non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations simultaneously. No protease inhibitor (PI)-related mutations were found. There were no statistically significant differences regarding CD_4 and CD_8 cell counts, increment in CD_4 cell counts, HIV RNA level, the frequency of HIV-1 drug resistance mutations across groups (P>0.05).

Side-effects that might be associated with HAART are listed in Table 3. Fourteen symptoms were considered as suspected side-effects associated with HAART, among which only fatigue and dizziness were statistically significant among groups. Subjects in Groups B and C had lower frequencies of fatigue and dizziness than those in Group A (P<0.05, respectively).

Table 3. Comparison of Frequency of Side-Effects among Groups [Case (%)]

Side-effect	Group A	Group B	Group C	χ^2	P
Fatigue	25 (25.0)	12 (12.0)	15 (15.0)	6.467	0.039
Difficulty in sleeping	23 (23.0)	20 (20.0)	19 (19.0)	0.529	0.768
Nausea/vomiting	12 (12.0)	8 (8.0)	9 (9.0)	0.992	0.609
Dizziness	11 (11.0)	3 (3.0)	3 (3.0)	7.982	0.018
Abnormal dream	10 (10.0)	7 (7.0)	8 (8.0)	0.611	0.737
Diarrhea	8 (8.0)	6 (6.0)	13 (13.0)	3.175	0.204
Headache	9 (9.0)	2 (2.0)	5 (5.0)	4.886	0.087
Rash	7 (7.0)	9 (9.0)	10 (10.0)	0.590	0.745
Other symptoms	13 (13.0)	9 (9.0)	12 (12.0)	0.862	0.650

Note: *P*-values were calculated using Kruskal-Wallis H test

DISCUSSION

CM has always been an important component of healthcare in China. Since 2004, the State Administration of CM has launched the National Free CM HIV/AIDS Treatment Program for patients with HIV infection and AIDS. To date, this program has provided free CM treatment for tens of thousands of patients. The pilot provinces involved are encouraged to develop their own formulas and strategies of CM to meet local clinical demands.

SLFZC and QDC are designed to treat different symptoms of HIV/AIDS patients based on the theory of CM. The present study showed that neither SLFZC nor QDC contributed to beneficial virological or immunological outcomes, suggesting that they may be less efficient than Tangcao Tablet (唐草片), the only herbal preparation approved by China State Food and Drug Administration to be used in the treatment of AIDS symptoms, which has been reported to be able to raise CD_4 cell counts.[10] Since SLFZC and QDC did not have a negative impact on virological or immunological response to HAART regimens, and they were not associated with HIV-1 drug resistance, it means that both preparations do not interfere with the efficacy of these HAART regimens. We also found that use of SLFZC or QDC was associated with lower frequencies of fatigue and dizziness that are considered as HAART-associated side-effects. However, they were not as potent as anticipated, because many other side-effects were not statistically significant across groups, especially those common symptoms such as nausea/vomiting, diarrhea, etc. These findings suggest that there are not many clinical benefits from the use of SLFZC or QDC.

It has been demonstrated that herbal extracts such as *Hyptis suaveolens* and *Myrothamnus flabellifolius* are potent inhibitors for cytochrome P450 (CYP450) enzymes,[6] leading to increased concentrations of antiretroviral drugs and increased risk of developing side-effects. In contrast, some herbs, such as *Sophora flavescens* and *Astragalus membranaceus*, can increase CYP450 activities,[11,12] leading to decreased concentrations of antiretroviral drugs and inefficient HAART. The concern that SLFZC and QDC might affect the antiviral efficacy of antiretroviral drugs arises from that *Astragalus membranaceus* is also included in the formulation of SLFZC and QDC. Fortunately, both herbal preparations did not have a negative impact on the efficacy of HAART, nor caused side-effects and toxicities.

One limitation of our study is that the observation period was only 1 year. The important endpoint event of AIDS, mortality, cannot be compared among different groups. Second, we cannot provide the direct evidences whether and how these 2 CM preparations influence the blood concentrations of antiretroviral drugs involved in this study. Third, to get a large enough number of participants, the study subjects were recruited from different hospitals, where bias may arise, especially information bias.

In conclusion, the uses of 2 CM preparations, SLFZC and QDC, do not have a negative impact on the efficacy of HAART. They are not as potent in reducing HAART-associated side-effects as anticipated, either.

Conflict of Interest

The authors declare that there is no conflict of interests.

Author Contributions

Su QJ performed the investigation and HIV drug resistance testing, and drafted the manuscript. Wu FS and Song C conceived and designed the study. Lu ZZ and Liu ZW recruited the patients, collected their clinical data and laboratory data about T cell subsets and HIV RNA. Xiao J reviewed the literature and edited the manuscript.

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