

Aining Granule Stabilizes the Decline of CD4 Cell Count in HAART-Receiving HIV/AIDS Patients Having Virologic Failure

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Abstract

Objective: To explore the pattern of slow-down of CD4 T cell depletion by Aining granule administration.

Method: The data of prospective, randomized, placebo-controlled and double blinded clinical trials enrolling one hundred HIV/AIDS individuals, randomized into two groups, one with 50 cases administered with Aining granule plus the combination of d4T, ddI and NVP, and the other received with placebo plus the combination of d4T, ddI and NVP for observing in a duration of 11 months in were re-analyzed to observe the course of different CD T cells over the treatment period.

Results: Only the patients in the Aining granule treatment group (7, vs 0 in the control group, deviation exceeding 2 sigmas) had stable CD4 T cell count over the treatment course.

Conclusion: Our results provided insights into molecular investigation of the relation between the active ingredients of Aining granule, DNA replication and HIV-induced CD4 T cell death.

Keywords: Aining granule; Cluster of differentiation; Highly-active antiretroviral therapy; Human immunodeficiency virus; Acquired immunodeficiency syndrome

Introduction

China and some developing countries still have vast regions that older regimen of Highly Active Antiretroviral Therapy (HAART) is being administered [1]. Moreover, the side-effects of HAART usually result in poor compliance [2]. According to UNAIDS 2012 China AIDS Response Progress Report, China has a population of 780,000 people living with HIV/AIDS (PLHIV). While HAART is the mainstream medicine in many countries, in China Chinese medicine has also been widely used as auxiliary medicine to ameliorate the side-effects of HAART [3-6]. Chinese medicine herbal formulas were found to be effective in promoting T lymphocytes either in the early or intermediate stages of HIV infection [7] or advanced AIDS [8], though the mechanisms remain to be elucidated. Due to social belief, HAART availability and toxicity considerations, Chinese medicine is also used solely by a significant number of PLHIV in China [9]. Previously we reported that combination therapy of HAART and Aining granule had resulted in a slower depletion of CD4 T cells and improvement in signs and symptoms, without any apparent adverse effects [10]. In spite of statistically significant results, we only compared time point to time point but did not take the course into consideration. Moreover, it is not known how the slowdown in CD4 T cell decline is related to the course. Bioinformatics techniques were used to explore patterns in various settings [11-15] and an in-depth pattern discovery shall shed light for better design of molecular experiments, drug administration and policy making.

Method

Patients met the inclusion criteria if they were absent of any other disease than HIV / AIDS. Otherwise they were excluded. A total of 100 patients with informed consent from Queshan County, Henan Province contracting HIV via compensated plasma donation infection had been recruited to a double blinded controlled study to compare the effect of HAART with and without Aining granule administration.

The patients were randomly assigned into the treatment group or the control group by central random voice systems at the clinical evaluation center of the Chinese Academy of Traditional Chinese Medicine. Patient demographics i.e. age and gender of the patients were averaged and recorded.

During an 11-month study period, the Aining treatment group was given Aining granule and HAART (in combination of d4T, ddI and NVP); the control group was given the HAART only. Both of the groups were inspected monthly by conducting routine blood, urine and stool tests at the clinical laboratory of Henan Province Queshan Hospital. CD4, CD8, CD3 and CD45RA cell counts at baseline and the 3rd, 6th and 11th month during the study period had been investigated.

Vacutainer blood collection tubes containing EDTA were used to collect blood samples. Peripheral blood mononucleated cells were prepared using conventional method and stored in liquid nitrogen before use. The stored samples were transferred to a 37°C waterbath for resuscitation. Phosphate buffered saline was used to rinse the cells twice before adding fluorescent labeled monoclonal antibodies for CD3, CD8, CD45 and CD4. The mixture was shaken and incubated at room temperature in the dark for 20 minutes, followed by addition of hemolysin. The samples were then shaken and incubated in the dark at room temperature for another 8 minutes. BD FACSC Calibur and

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Received November 28, 2014; **Accepted** January 31, 2015; **Published** February 07, 2015

Citation: Ross LK, Frances LFS, Yu-wen C, Yang X, Jian W (2015) Aining Granule Stabilizes the Decline of CD4 Cell Count in HAART-Receiving HIV/AIDS Patients Having Virologic Failure. J Data Mining Genomics Proteomics 6: 165. doi:10.4172/2153-0602.1000165

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FITC-IgG1/PE-IgG1/PCy5-IgG1 were used for cell sorting. For each sample at least 10,000 cells were used for detection. Aining granules had been procured from Shanghai Sunshine Biotechnology Ltd. (batch number: 2005L01872). Patients with records of poor compliance and missing any records at 0, 3, 6 and 11 months during the study period were excluded for further analysis.

Statistical analysis by either Wilcoxon rank sum test with continuity correction or Fisher's exact test, wherever appropriated, was performed. Self-organizing map was generated by R (version 2.15.2) to demonstrate the change of various CD T cells count over the study period. Eight clusters were used to classify the patterns in the course of CD4, CD3, CD8 and CD45RA T cells to represent increase or decrease for the transition between 0, 3, 6 and 11 months after the initiation of HAART (and Aining treatment in the treatment group).

Results

The data of previously recruited 100 HIV/AIDS patients were retrieved. Six of them manifested very poor compliance and were excluded from subsequent analysis. Only 33 patients in Aining granule treatment and 24 patients in control groups contained complete information in the CD4, CD3, CD8 and CD45RA T cell course. The details of demographical and clinical information are summarized in Table 1.

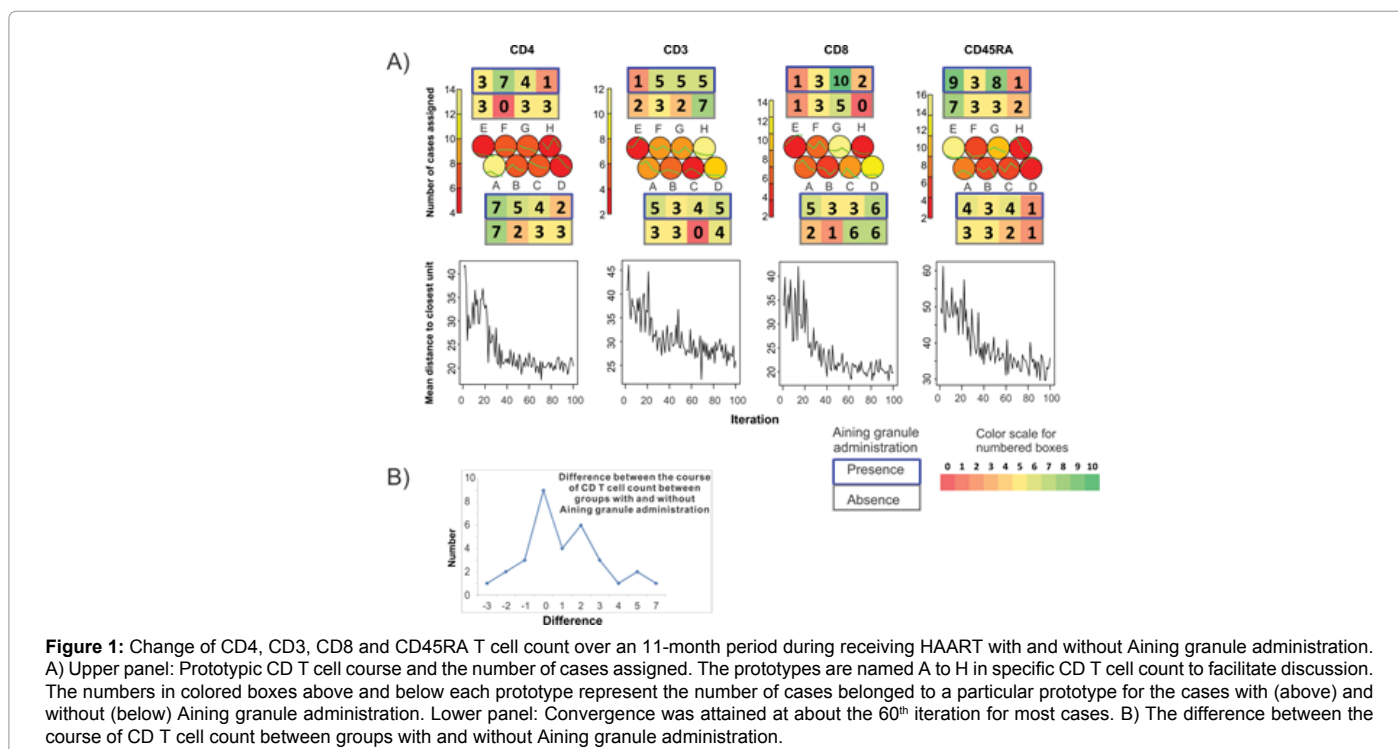
All the attributes were not significantly associated. The decline in CD4 T cell counts at baseline and 11 months post-HAART with (-72.5) and without Aining granule administration (-119) was significant (U-test, 0.044), a result comparable to our previously reported values [10]. Figure 1A shows the course of CD4, CD3, CD8 and CD45RA T cells. There were no apparent differences in the course of specific CD T cell change for the two groups, except that CD4 T cell count remained stable for 7 HIV/AIDS patients in the Aining granule treatment group (Figure 1A, CD4, prototype F). Since there were 33 and 24 patients in the Aining granule treatment and control groups,

respectively, an assumption of random distribution of the two groups over the CD T cell count will yield an average difference of $33/24=1.375$. In other words, 0-2 more patients having received Aining treatment is expected. In fact, Figure 1B supports this idea and a normal distribution was observed. Double peaks are expected because all the differences were integer. The extreme value of 7 (deviation exceeding 2 sigmas) again suggests that CD4 T cell count remaining stable might be the major effect of Aining granule. The courses of CD3 and CD8 T cells were similar (Figure 1A) and these were also supported by high Spearman's rank correlation coefficients (Table 2).

Discussion

Virologic failure is particularly problematic in rural areas due to poor compliance [16]. Although the emergence of HIV drug resistance (HIVDR) also poses a threat to the long-term success and durability of HAART [17], a significant proportion of patients in virologic failure had no drug resistance identified in a large cross-sectional cohort study in China, suggesting that treatment adherence could contribute significantly to the success of treatment [18].

Our results showed that over the 11-month treatment period, only the CD4 T cell count had a significant difference between Aining administration and control group. The decline was also not retarded immediately, but after a period of 11 months, in concordance with our previously published result [10]. Here we provided additional evidence that the slowdown was partly contributed by a group of patients that managed to maintain CD4 T cell count. Collectively, Aining granule might play a role in stabilizing CD4 T cell count in some patients while slightly slowing down the decline of CD4 T cell count in others over an extended period. By dissecting the course of different CD T cells over the treatment period, we also showed that there was no difference between the two groups in CD T cells other than CD4, implying CD4 T cell regeneration (reflected by CD45RA cell count) was not affected. CD8 cells were also found not significantly influenced by the Aining granule. During the course of AIDS progression, CD4 T cell counts



Category	Frequency/Median		p-value#
	with Aining	without Aining	
Gender			
Male	13	5	0.161
Female	20	19	
Age	43	39	0.082
CD4 count (months after treatment)			
baseline	278	339.5	0.485
3 months after HAART	317	321.5	0.841
6 months after HAART	254	286	0.626
11 months after HAART	227	218	0.968
Annual difference	-72.5	-119	0.044
CD8 count			
baseline	880	735.5	0.626
3 months after HAART	848	586.5	0.258
6 months after HAART	847	686	0.516
11 months after HAART	675	625	0.536
Annual difference	-154	-135	0.828
CD3 count			
baseline	1130	1162.5	0.865
3 months after HAART	1113	923.5	0.604
6 months after HAART	1217	1122	0.893
11 months after HAART	934	913.5	0.879
Annual difference	-197	-272	0.390
CD45RA count			
baseline	1552	1504.5	0.892
3 months after HAART	1603	1169	0.465
6 months after HAART	1442	1616.5	0.66
11 months after HAART	1228	1220	0.765
Annual difference	-303	-361	0.456
#Wilcoxon rank sum test with continuity correction and Fisher test were performed where appropriate			

Table 1: Summary of patient characteristics

Time point	Spearman's rank correlation rho	p-value
baseline	0.921	<0.001
3 months after HAART	0.911	<0.001
6 months after HAART	0.950	<0.001
11 months after HAART	0.918	<0.001

Table 2: The correlation between CD3 and CD8 T cell count during the course of treatment

decreased while the other CD T cell counts were less affected, it is expected that the ratio of CD4:CD8 should also follow the decreasing trend. Since the biomarker CD3 actually mainly represents CD3+CD4+ and CD3+CD8+ T cells, a strong correlation between the course of CD3 and CD8 T cell change over the 11-month treatment period was observed.

Recently, DNA-dependent protein kinase has been identified as a potential modulator in HIV-1 induced CD4 cell death [19]. Radix Astragali, a major ingredient of Aining Granule, was shown to induce T lymphocytes cell proliferation and phosphorylation of extracellular signal-regulated kinase [20]. Ginseng is another major ingredient in Aining Granule that was reported for slowing CD4 T cell depletion in HIV-infected patients [21]. Another study showed that ginseng can promote CD4 T cell proliferation and dendritic cell stimulation [22]. Although Chinese medicine is a complex mixture of heterogeneous ingredients, considering social belief, HAART availability and toxicity considerations, the beneficial effects of Chinese medicine should be further explored to improve the quality of life for the PLHIV in China.

Acknowledgement

This work was supported by Stanley Ho Centre for Emerging Infectious Diseases and Core Laboratory facilities, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong. We thank Liu Ying for her coordination.

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