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PHASE-1 STUDIES OF MALARIOOTHERAPY FOR HIV INFECTION

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Objective. To determine whether malariortherapy (an old therapy for treatment of neurosyphilis) improves some clinical and laboratory parameters of HIV-positive patients without iatrogenic complications.

Methods. Total 8 asymptomatic HIV-1 positive subjects whose CD4 cell counts were over 250×10^6 cells/L were selected for the phase-1 studies of malariortherapy and were intravenously injected *Plasmodium vivax* to induce artificial malaria. Malaria was terminated with chloroquine after 10~20 malarial fever episodes. Cell-bound CD4 levels were measured by APAAP (a solid-phase enzyme assay) and levels of neopterin (NPT), beta-2-microglobulin (B2M), soluble tumor necrosis factor receptor-2 (sTNF-RII), interleukin-2 (IL-2) and HIV P24 antigen were measured by ELISA. Patients were followed up to 24~30 months.

Results. CD4 levels increased in 5, NPT decreased in 7 of 8 patients; IL-2 increased in 5 of 6 patients after malariortherapy. The total trends of B2M and sTNF-RII basically remained stable. HIV P24 antigen remained undetectable in 6, remained detectably low level in 1 and experienced increase in 1 of 8 patients after malariortherapy. No any severe complications occurred in all 8 patients.

Conclusions. The results indicate that malariortherapy basically is safe for HIV infection and it seems that the therapy improves some immunological parameters of HIV patients.

INTRODUCTION

Since the first case of acquired immunodeficiency syndrome (AIDS) was found in 1981, no curative therapy for AIDS and for human immunodeficiency virus

(HIV) infection has been yet established. Drug combination therapy or highly active antiretroviral therapy (HAART) can kill HIV enormously in plasma and in activated CD4 lymphocytes therefore can lower HIV viral load to undetectable and then restores a part of immune function, but HIV in resting memory CD4 cells and in other virus reservoirs such as in lymphoid tissues and central nervous system can not be eliminated (1,2). Therefore drug therapy eventually faces intolerable side-effect or toxicity due to long term treatment. In addi-

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tion, HIV/AIDS patients account for over 90% in the world living in developing countries are unable to afford the drugs since the prices are too high to them. Immune-based therapy such as interleukin-2 (IL-2) therapy has been tried and has shown some effectiveness in restoring immune function of HIV/AIDS patients (3, 4). But IL-2 therapy induces variable side-effect including fatigue, right upper quadrant pain, abnormalities of gallbladder and other symptoms or complications (5,6) and also, it can not kill HIV *in vivo*. So it is still far from the goal of cure of HIV/AIDS treated either with HAART or with immune-based therapy. The idea of trying malariotherapy for HIV/AIDS came to us (7) when we got the following data: 1. 71 AIDS patients died at the rate of 35%, but 41 AIDS patients coinfecting with malaria, nobody died during the same observed period. 2. In Venezuela, Indonesia and the Philippines, in malarial endemic regions, HIV-like antibodies existed, but no AIDS patients in these regions and AIDS did exist in nearby non-malarial areas. 3. Malaria does not accelerate HIV infection progress and neither HIV infection deteriorate malarial patients. 4. Malariotherapy had been successfully used to treat neurosyphilis since 1917, its discoverer, Dr. Wagner-Jauregg therefore won the Nobel Prize of Medicine in 1927. 5. Safety of malariotherapy has been confirmed in treating neurosyphilis in the past decades.

Through a couple of institutional board discussions, a final approval of phase-1 studies (no more than 10 cases) of malariotherapy for HIV infection conducted in Guangzhou was obtained from the board.

MATERIAL AND METHODS

Patient selection and malaria induction. Total 8 asymptomatic HIV-1 positive (confirmed by Western Blot) patients with CD4 counts over 250×10^6 cells/L, who were naive of any kinds of antiretroviral therapy were selected for the phase-1 studies of malariotherapy. All patients are male; ages of case 1~8 were 23, 40, 34, 29, 27, 31, 33 and 31 respectively. Case 1~2 got HIV from sexual transmission and case 3~8 from injecting drug use (sharing needles). All patients signed informed consent. Plasmodium vivax (Pv.) malarial blood (10 ml, results of all tests for HIV, hepatitis B and syphilis were negative) was intravenously injected into HIV patients. Malaria was terminated with chloroquine after 10~20 malarial fever episodes.

Laboratory testing. Cell-bound CD4 levels were determined by the APAAP method (a solid-phase enzyme assay, the kits were manufactured by Chinese Military Medical Scientific Academy) in Guangzhou, China. The levels of soluble immune factors in serum including

neopterin (NPT, kits of Diagnostica GMBH, Germany), beta-2-microglobulin (B2M, Diagnostica GMBH), soluble tumor necrosis factor receptor-2 (sTNF-RII, Genzyme Corporation, USA), IL-2 (Genzyme Corporation) and HIV P24 antigen (immuno-complex dissociation method, abbreviated to ICD, Coulter Corporation, USA) were measured by ELISA in the University of California at Los Angeles (UCLA), USA.

RESULTS

Clinical response. Malarial fever episodes and parasitemia began after 1~3 weeks of Plasmodium inoculation. Patients got every day or every other day febrile paroxysm. All patients remained asymptomatic of HIV infection and felt stronger after termination of malaria. Light anemia occurred during malarial phase, but all recovered to baseline within three weeks after termination of malaria without blood transfusion. Livers and spleens were slightly larger than the baseline during malarial phase, but also recovery within three weeks after cure of malaria. Malaria manifestations seemed slightly milder (at least not more serious) than that in those patients with vivax malaria who were HIV negative according to our previous clinical experience. There were no any severe complications occurred in all 8 patients. Malaria in all 8 patients was very sensitive to chloroquine, all cured of malaria after one course of 10 tablets (one tablet contains 0.15g matrix of chloroquine) treatment, no recrudescence or relapse occurred.

Immunological parameters. CD4 dynamics; 5 (case 1, 2, 4, 5, 7) of 8 patients experienced CD4 increase, other 3 patients (case 3, 6, 8) remained CD4 levels over 500×10^6 cells/L after malariotherapy and followed up to 18~30 months (Table 1). Total trend of NPT decreased to lower than baselines in 7 of 8 patients, recov-

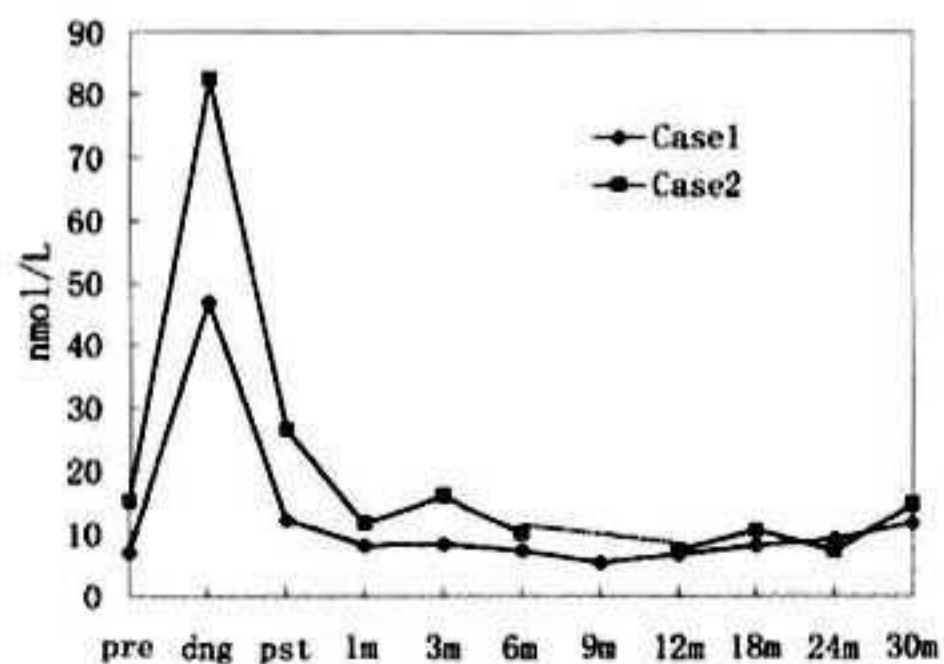


Fig 1. NPT dynamics in HIV patients infected with malaria (case 1,2) (dng:during malarial phase)

ered to baseline in one patient (case 1) during period of follow up(Figs 1,2). B2M levels remained stable in 6

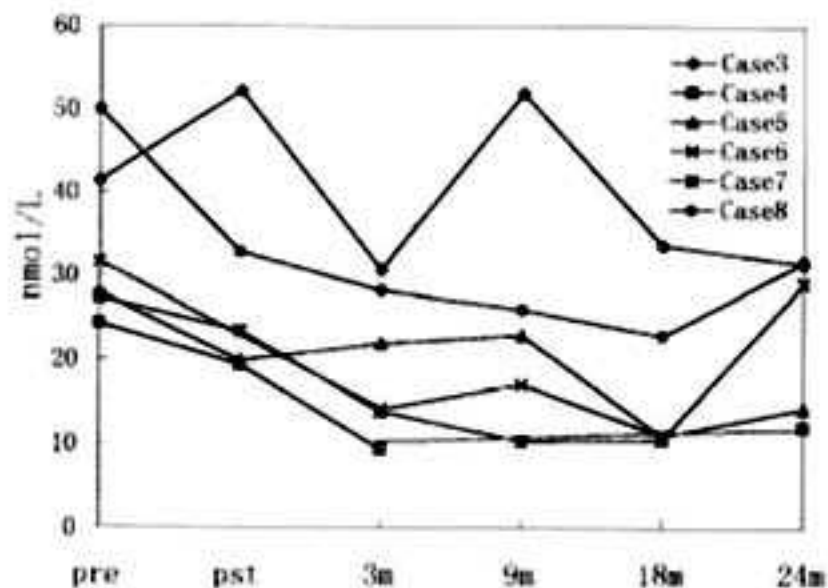


Fig 2. NPT dynamics in HIV patients infected with malaria (case 3~8)

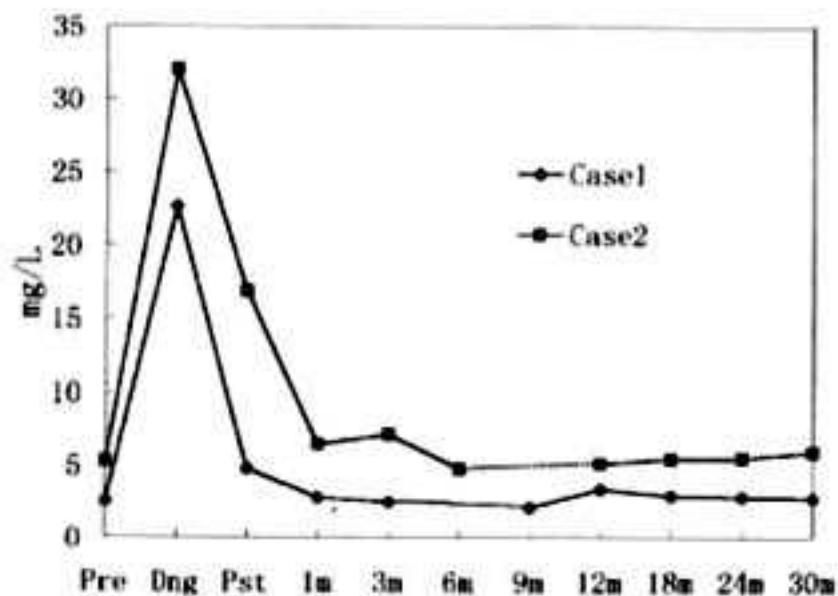


Fig 3. sTNF-RII dynamics in HIV patients infected with malaria(case 1,2)

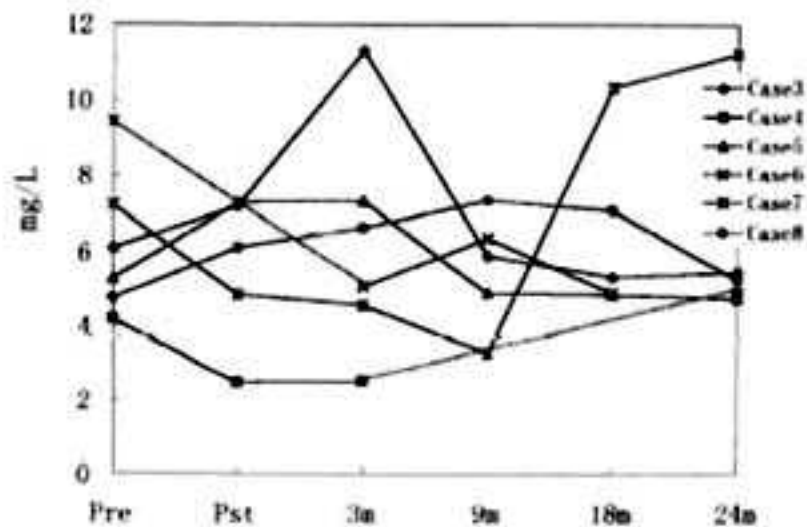


Fig 4. sTNF-RII dynamics in HIV patients infected with malaria(case 3~8)

(case 3~8) of 8 patients; the levels decreased in one patients(case 1) at the time point of 3rd month follow up and then increased to over baseline after 12th month

follow up; one patient (case 2) underwent decrement after treatment and continued to 24 months follow up (detail data not shown). Total trend of sTNF-RII levels in 6 of 8 patients basically remained stable except fluctuation of case 3 and 7 (Figs 3,4). We tested NPT, B2M and sTNF-RII during malarial phase in case 1 and case 2. All these three immune-related factors increased to very high levels even though they sharply decreased to baseline level (case 1) and to lower than baseline (case 2) after malariotherapy and during period of follow up. IL-2 levels were measured in 6 patients at pre-treatment and post-malaria, 5 (case 2~5 and 7) experienced apparent increase and one (case 1) remained undetectable(Fig 5).

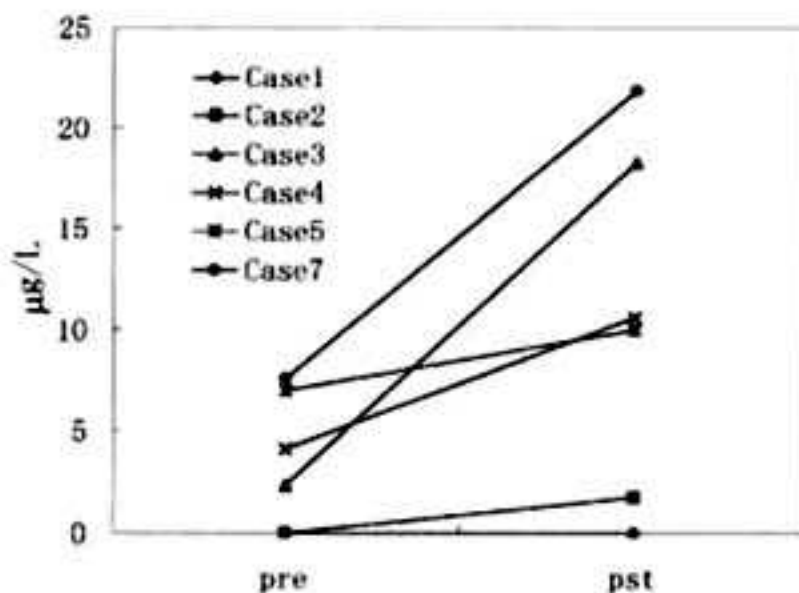


Fig 5. Changes of IL-2 levels in HIV patients at pre- and post-malariotherapy

HIV P24 antigen. Among 8 patients, 6 remained undetectable of HIV P24 antigen at all testing time point. One patients (case 7) remained detectably low levels at least up to 18 months follow up; one patient (case 2) underwent increment of P24 antigen but interestingly, this patient got the most apparent elevation of CD4 levels in the studies(Fig 6 and Table 1).

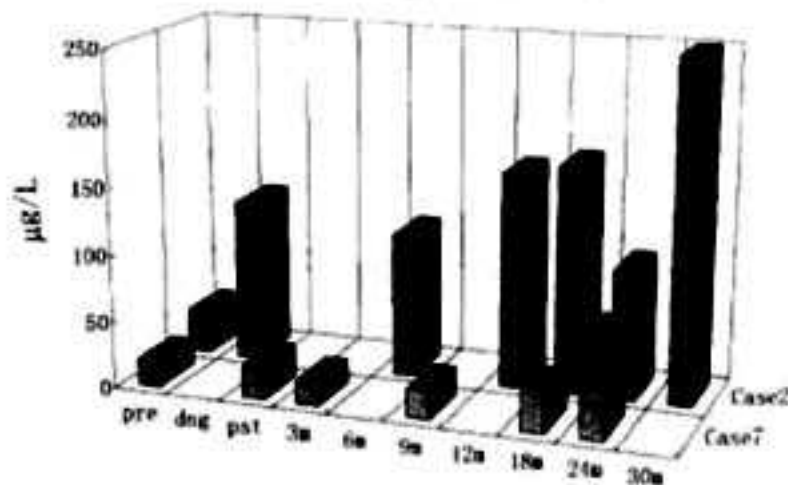


Fig 6. HIV P24 dynamics in HIV patients infected with malaria(case 2,7)

Table 1. Dynamics of CD4 counts ($\times 10^6$ cells/L) of HIV patients infected with malaria

Case No.	1	2	3	4	5	6	7	8
pre	889	269	1610	731	1868	1056	924	705
pst	853	527	979	1183	1248	1000	600	826
1m	936	469						
3m	1260	626	1690	1813	2052	1384	1152	1922
6m	1162	953						
9m			750	1631	1813	875	1260	572
12m	1258	656						
18m	1072	864				665		840
24m	941	650	1151	2063	2422		1081	570
30m	1697	1199						

Note: pre = pre-malariotherapy; pst = post-malariotherapy; m = month(s) of follow up

DISCUSSION

A lot of reports have shown that malaria stimulates immune system to produce a variety of immune factors including IL-1, IL-4, IL-6, TNF- α , IFN- α , IFN- γ (8), IL-2R(9) and sCD8 (10) and increase of many of these factors is harmful to HIV/AIDS patients (such as promotes HIV replication) based on the data(11,12) of HIV/AIDS research. This was why many doctors including American and Chinese doctors opposed us when Dr. Henry J. Heimlich first proposed the idea of malariotherapy for HIV/AIDS early in 1992(7). In a couple of institutional board discussions on this issue, many criticisms came from the board members and during the time, Dr. Xiaoping Chen proposed a hypothesis to discuss with those who opposed us that all these harmful factors would do increase during malarial phase based on our previous knowledge on malaria, but after termination of malaria, these factors would sharply recover to baseline and even to lower than baseline due to a feedback mechanism of immune system. The board finally approved us to treat no more than 10 HIV patients with the therapy in the phase-1 studies.

It was clear in our studies that some immune substances (activation markers) such as NPT, B2M and sTNF-RII(13) did significantly increase during malarial phase and then sharply decreased to around baseline (B2M and sTNF-RII) and to lower than baseline (NPT) after malariotherapy.

A very important fact was that even though NPT, B2M and sTNF-RII were very high during malarial phase, there were no any severe complications occurred in all 8 patients and no any evidences to show enhance-

ment of HIV replication because HIV P24 antigen did not significantly elevate in 7 of 8 patients within the period and hereafter. Very interestingly, it seemed that manifestations of vivax malaria in these HIV positive patients were slightly milder than that in those HIV-negative subjects according to our previous clinical experience. In an animal model study(14), HIV-like virus infection significantly diminished the gravity of neurological manifestations and therefore decreased the mortality of malaria in mice. We have not yet completely understood the mechanism that why HIV-like virus infection prevents death of animal from cerebral malaria, but intriguingly, this kind of protection induced by murine AIDS (MAIDS) was increasingly strengthened with the severity of immunodeficiency, which was identical with the levels of IL-10 secreted by T lymphocytes. As reported, IL-10 was considered as a counter-acting cytokine against IFN- γ and TNF- α (secreted by macrophages) which play an important role in pathogenesis of cerebral malaria.

It is too early to say that malariotherapy prolongs life of HIV patients, but in our 8 patients, 5 experienced CD4 increase, 3 remained CD4 levels over 500×10^6 cells/L and 7 underwent NPT decrease up to 18~30 months follow up. Only one patients (case 6) died at the time point of 24th month follow up (but not died of AIDS based on investigation of his medical history). Other 7 patients remained clinical well 24~30 months after malariotherapy. Some reports indicated that CD4 elevation plus NPT reduction was a better parameter of effectiveness of treatment than decrease of single HIV viral load level(15). In another study(14) of murine models coinfecting with MAIDS and malaria, Plasmodium infection delayed progression of MAIDS instead of promotion which was expected. Not only did malarial parasite infection in profoundly immunosuppressed mice improve manifestations of MAIDS, but also a very significant pathological effect namely 50% weight loss of lymph nodes was observed, which was associated with shrinking of B lymphoma nodules. We could not confirm the same change occurred in our 8 HIV patients due to the difficulty of precise measurement of lymph nodes even though we felt their decrease in size in our visits of the patients during and after malariotherapy, but they felt stronger after the therapy implied that some similar pathological improvement might occur in our HIV patients.

Increase in IL-2 levels in 5 of 6 patients were identical with the total trend of increase of CD₄ levels (5 of 8) after the malariotherapy. Some studies in vivo and in vitro showed that HIV infection stimulated production of IL-1, IL-3, IL-4, IL-6, IL-10, TNF- α , TNF- β ,

IFN- α and IFN- β and suppressed production of IL-2, IFN- γ and IL-12 (11, 12). In MAIDS models, a progressive imbalance in cytokines of Th1 (IL-2, IFN- γ and IL-12) to Th2 (IL-4, IL-6 and IL-10) was confirmed and the imbalance delayed while coinfecting with malarial parasites (14). It seems that HIV similarly triggers a change from Th1 to Th2 pattern of cytokine response and in the contrary, malaria may call back a shift from Th2 to Th1 pattern. This will be confirmed or overthrown in our coming study.

The result of that HIV P24 antigen levels did not apparently increase in 7 of 8 patients was consistent with indifference in viral load levels between MAIDS models and animals coinfecting with HIV-like virus and Plasmodium (14). These results indicated that malariotherapy did not significantly enhance HIV replication while stimulating immune system to produce more IL-2 and CD4 cells.

In conclusion, through the phase-1 studies we have preliminarily confirmed that malariotherapy basically is safe for HIV infection. It seems that the therapy improves some immunological parameters of HIV patients and the mechanism may be due to stimulating production of IL-2 or triggering Th1 pattern of cytokine response and/or blocking apoptosis of CD4 lymphocytes. It also seems that it can not kill HIV in vivo as the way as anti-retroviral therapy but there may be a "cook effect" (periodical high fever episodes) combining with immunological effect or pressure on HIV to induce change from syncytia-inducing (SI) to non-syncytia-inducing (NSI) phenotype (11) or from high toxic virus strain(s) to low toxic strain(s). All these will be further determined in our coming study.

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