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The Novel Study of IMOD™ against HIV-1, P24 production

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ABSTRACT

AIDS (Acquired Immune Deficiency Syndrome), a result of human HIV (Human Immunodeficinency Virus) infection, is one of the most troublesome world's health problems. Extensive researches to find effective drugs for its treatment are running fast in huge capacities. IMOD (Immuno-Modulator Drug) is the name of an herbal drug that has modulatory effects of immune system. As a goal of this research, IMOD was tested to determine its effect on HIV-infected cells, and whether it can inhibit viral P_{24} gag protein production or not. Human PBMCs (Peripheral Blood Mononuclear Cell) isolated from peripheral blood by Ficoll-gradient centrifugation from two groups including 15 HIV-infected patients and 5 non-infected persons as the control group. Then the cells were cultured and the plates were incubated for 3 days in a humidified Co_2 incubator at 37°C. Then IMOD and AZT (Zidovodine) were added to the progeny of each flask. After 48 hours, the amount of P_{24} in supernatant was evaluated by Enzyme-linked immunosorbent assay (ELISA). It was demonstrated that the concentration of P_{24} in AZT treated flask was low but in IMOD treated flasks was variable. Our study reveals that IMOD couldn't inhibit P_{24} production in HIV positive individuals, although this conclusion is only based *in vitro* study and specific research which must be tested *in vivo*. In conclusion, although AZT group has higher viral load but it can release P_{24} antigen more than IMOD treated groups.

1. Introduction

One of the main threats to human existence and health worldwide is AIDS that is caused by Human Immunodeficiency Virus (Ghoneum et al., 2010). More than 22 million people have died and an estimated 50-70 million are living with HIV (Kilmarx et al., 2009). CD_4 T cells are an integral part of the antiviral response (Moss et al., 2000a). An increase in immunodeficiency and the transition to AIDS is marked by a decrease in the number of CD_4 T lymphocytes (Moss et al., 2000b). On the other hand it can be expressed when the number of CD4 T cells is less than 200 per micro litter of blood and the person with an antibody against various operating components of the AIDS virus in the body is to be identified as a person with AIDS (Temmerman et al., 1995). Extensive researches on

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the effective anti-HIV drugs have been done and this leads to introduce various drugs that inhibit the virus. But these drugs could not eradicate the virus from the body (Kerouedan et al., 2006; Buchbinde et al., 2009). A new drug, Setarud (IMOD), with the capacity to correct immune deficiencies was introduced for the treatment of HIV infection. It is a mixture of herbal extract, enriched with selenium. It has been demonstrated that Setarud has positive effects on the immune system when administered to animals (Heshmat et al., 2007). The IMOD ™ extraction can be used as a first step in treating and managing HIV-positive patients. From another perspective the long–term effects of this combination in most patients can be exploited as a therapeutic vaccine (Khairandish et al., 2009; Mohraz et al., 2007; Neumann et al., 1999).

The aim of this study was to demonstrsate the effects of IMOD on HIV-infected cells, and to determine whether it can inhibit viral P_{24} gag protein production.

2. Materials and Methods

2.1. Study design and patient

HIV-1 chronically infected patients who had been seropositive for 7 to 11 years received a HAART (Highly Active AntiRetroviral Treatment) regimen with 2 nucleoside analogs plus one protease inhibitor or one nonnucleoside reverse transcriptase (RT) inhibitor. The patients who had the CD4 lymphocyte count of 400/mm3 and the plasma HIV RNA count of 200 copies/ml for at least 6 months were recruited for the study. All signed an informed-consent form based on our previous observation (Li et al., 1998). One of the objectives of this study was obtaining cells from Iranian HIV-positive patients. For this purpose a number of HIV positive patients from AIDS research center of Imam Khomeini hospital in Tehran were selected. After obtaining written consent from the patients, 4 ml of fresh blood was taken from each case in sterile tubes containing EDTA (Ethylenediaminetetraacetic acid) anticoagulant. The total numbers of 15 patients with HIV were included in this study.

2.2. Principles of the procedure for CD_4 counting and viral load; Flow cytometry

Absolute CD_4 cell counts were obtained in fresh blood samples as described in protocol (Schinazi et al., 2001). Quantification of HIV-1 RNA levels in plasma was performed using the Amplicor Monitor assay (Roche Diagnostics, Basel, Switzerland) with limit detection of 200 copies in real time (Kheirandish et al., 2007).

2.3. PBMCs isolation

Preparation of peripheral blood PBMCs were presented from heparinized peripheral venous blood by ficoll-hypaque density gradient centrifugation. Blood (4 ml) was gently overlaid on the surface of 2 ml ficoll-hypaque and centrifuged at 7000g for 20 minutes, and then buffy coat which contained PBMCs was aspirated using Pasteur pipette. The cells were washed twice with HBSS (Hank's balanced salt solution) and re-suspended in serum-free medium (GIBCO, Long Island, NY).

2.4. Viability

The cell viability was measured with 0.4% trypan blue which mixed with cell dilution solution and was counted with hemacytometer slide under a binocular microscope. The unstained (viable) and stained (nonviable) cells were counted separately.

2.5. Cell culture

Normal peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation process from HIV-1 seropositive donor and seronegative donor as described earlier. PBMCs (3×10^6 cell per ml) were cultured in RPMI 1640 medium (GIBCO, Long Island,NY) containing 10% fetal bovine serum, Penicillin 100 U ml^{-1}, Streptomycin 100 mg ml^{-1} and 2 µml Glutamine mediums (Gibco-BRL, Gaithersburg,MD) and incubated at 37°C.

2.6. IMOD titration

To obtain proper dilution of the drug, tests were evaluated independently, to get appropriate medication that the lowest headline rate of cell toxicity. The isolation of PBMC cells from 10 healthy individuals (apart from the groups when evaluating the effect of a drug) was performed

according to procedure. These cells were diluted after counting and the viability of cells at different time in the vicinity of various drugs was evaluated. After that, the samples were taken from each flask and the number was assessed. In addition the amount and cell viability of each flask was examined.

2.7. Injection and production of HIV-1 P_{24} antigen

Four days after culture, IMOD was injected to the cell culture flask. Another set of experiments was carried out to evaluate the effects of AZT in the amount of 1 nmol/ml. At the end of the incubation period (48 hour), culture supernatants of HIV-infected cells were collected and analyzed for viral production. HIV-1 P_{24} antigen was assayed using a commercially available ELISA kit, (DuPont NEN, Boston, MA, USA) according to manufacturer's protocol.

3. Results

3.1. Virus-RNA and CD_4 counting

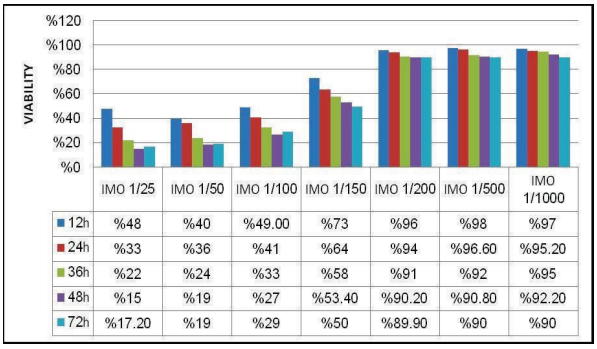
Table 1 summarizes the patients' characteristics. Range of CD_4 counting was between 100-500 and RNA copy number was more than 4000 copies (table 1).

Table 1. CD_4 and viral load numbers for each patient

Specimen ID	Viral Load	CD_4 Counting
4	850390	220
5	900820	416
6	1500000	179
7	1120000	131
8	750000	315
9	100000	290
10	25600	400
11	790000	187
12	50825	310
13	49300	275
14	500436	415
15	40620	340
16	1231000	310
17	720000	289
18	1150000	145

3.2. IMOD titration

The concentration of IMOD for this study has been determined. The concentration of the IMOD is very important and sensitive. According to the results obtained in this study, the rate of cell survival in the concentration of 1/200 within 48 hours was 90.20% (Figure 1) which was the highest viability.



	IMO 1/25	IMO 1/50	IMO 1/100	IMO 1/150	IMO 1/200	IMO 1/500	IMO 1/1000
12h	%48	%40	%49.00	%73	%96	%98	%97
24h	%33	%36	%41	%64	%94	%96.60	%95.20
36h	%22	%24	%33	%58	%91	%92	%95
48h	%15	%19	%27	%53.40	%90.20	%90.80	%92.20
72h	%17.20	%19	%29	%50	%89.90	%90	%90

Figure 1. Survival rates of cells in different dilutions and times of IMOD administration in this study

3.3. P24 measurements

As described above, measurements of P_{24} were done by anti P_{24} ELIZA kit. The results show that the average concentration of P_{24} for the first passage is less than the second passage, and the second passage is less than the third one (Figure 2). As mentioned above, we had 3 groups in this study: IMOD treated group, AZT treated groups and no treat group. It has been shown that there is a relation between the type of medicine and P_{24} concentration. It has been shown that P_{24} concentration in no drug flasks was higher than IMOD treated flask, and IMOD treated were higher than AZT one. It was shown that P24 concentration for the groups with no drug was higher than IMOD and AZT treated groups.

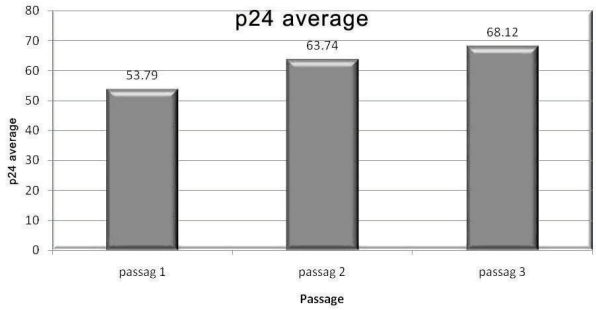


Figure 2. The average concentration of P_{24} was compared in three passages.

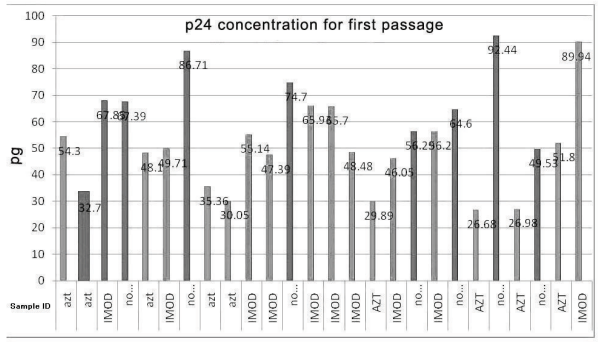


Figure 3. P24 concentration for the first passage in different medicine administration. As it is shown, the highest concentration was seen in the groups with no injection

3.4. Statistical Analysis

In order to compare the P_{24} concentration Manvitny Test was used. A significant difference between the groups has been shown. AZT could reduce P_{24} more effectively than IMOD (Table 2). The data were analyzed using SPSS software version15.

Table 2. The statistical analysis of the data

	IMOD(9)	AZT(6)	PV
V.L.	170000±3000	930000±2000	0.008
CD_4	265±88	280±96	0.75
OD	1.08±0.32	0.708±0.34	0.05
P.g. P_{24}	55.5±17.3	38.4±15.9	0.06

4. Discussion

The results of this study showed that IMOD as an herbal extraction anti-HIV drug caused a significant decrease in the production of P_{24} antigen. Recently, there has been much research on the immune system modulator drugs.

P_{24} antigen is the principle core protein of HIV and is found in serum either free or bound with anti-P_{24} antibody (AmpliPrep/COBAS TaqMan HIV-1), (Povolotsky et al., 1998). Virus levels in the peripheral blood can be analyzed by measurement of the HIV P_{24} antigen in serum using quantitative culture of HIV from plasma (Ghoneum et al., 2010; Finzi et al., 1999). The growth of virus was detected by measuring of P_{24} antigen in culture supernatants using ELISA (Gervaix et al., 1997; Turk et al., 2002). In our experiments based on P_{24} release into culture supernatant reveled that AZT is more potent than IMOD for inhibition of P_{24} production in HIV infected cell culture.

There was a significant difference in the P_{24} concentration between AZT and IMOD groups ($P<0.05$). AZT belongs to the NRTIs (Nucleoside analog reverse transcriptase inhibitors) group. AZT effectively inhibits the replication of HIV-1 (Sharipova et al., 1997). Although numerous papers have been published regarding P_{24} antigen as a prognostic marker for HIV infection but there are little data which address the relationship between P_{24} antigen and production of CD_4 lymphocytes (Carcelain et al., 2001; Heshmat et al., 2008; Mohraz et al., 2009).

CD_4 T cells are an integral part of the antiviral response. Recently, Th_1 (T-helper) immune response to core proteins (P_{24}) has been shown to be associated with control of viremia and better clinical outcomes (Moss et al., 2000a).

In the other hand, developing immune-based therapies indicate specific T-cell responses for the treatment of HIV. Setaurd, one of the IMOD components, may exert anti-viral and immune-stimulating properties (Rua Micheletti et al., 2009). In one study, the magnitude of CD_4 T cell proliferative responses against HIV-1 Ags has been inversely associated with HIV-1 viral loads in some groups of HIV-1 infected individuals (Wilson et al., 2003).

On the other side, if the viral load measurement is high, it indicates that HIV is replicating and that the disease will likely progress faster than if the viral load is low (McArthur et al., 1997; Chaisson et al., 1999). In conclusion, although AZT group has higher viral load but it can release P_{24} antigen more than IMOD and act stronger.

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