

Full Length Research Paper

A novel approach to inhibiting human immune deficiency virus (HIV-1) infection by actively neutralizing the antibodies of reverse transcriptase system

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Human immune deficiency virus (HIV) infection is frequently reported in Egypt. This study introduces a new approach for HIV eradication based on a new enzyme combination reverse transcriptase and DNA polymerase (VK 25 RD) formula for inhibiting and or preventing the disease. This pilot study was done on five naive patients who were all positive for HIV antibodies, never treated with anti retroviral medications. Those patients were registered and under surveillance by Human immune deficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) Control Department at the Egyptian Ministry of Health (MOH). Their immunological data revealed a viral load of more than 1,000 copies/ml by human immune deficiency virus-ribonucleic acid-polymerase chain reaction (HIV-RNA-PCR), and antibody positive to HIV-1 and CD4+ T-cell values less than 250 cells/ μ l. All of the patients showed the same clinical symptoms of HIV/AIDS and wrote consent of acceptance to take this combination therapy in the form of subcutaneous injection of 0.1 cc twice daily for 24 weeks. At the end of therapy, all of the patient's viral loads had reached under the detectable limits (less than 16 copies/ml); also there were significant increases of their CD4 cells count over 500 cells/ μ l. According to these findings, this therapeutic modality was promising for treating HIV-1 disease and human immunodeficiency syndrome.

Key words: (VK 25 RD) Reverse transcriptase (RT), DNA polymerase mixture formula, acquired immune deficiency syndrome (AIDS), Human immune deficiency virus (HIV).

INTRODUCTION

In Egypt where this study is conducted, the prevalence of newly diagnosed human immune deficiency virus (HIV) positive patients is high, therefore Egypt belongs to one of the regions of the world with rising HIV epidemics. The virus is transmitted mostly sexually in 71% of the cases, with heterosexual contacts comprising almost half of all

detected cases. Intravenous drug use and blood transfusion each accounts for 9% of the detected cases, while the percentage of the transmission through renal dialysis and unknown modes comprise the rest.

HIV is a retrovirus that infects the human immune system, 'reverse transcriptase enzyme' is an essential

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part of the virus that reads the sequences of viral RNA nucleic acids that have entered the host cell and transcribes the sequences into complementary DNA sequences. HIV-1 reverse transcriptase (RT) is composed of an extended, asymmetric heterodimer of two related chains, a 51 kD subunit (p51) of 440 amino acids and a 66 kD subunit (p66) of 560 amino acids (Misra and William, 1999). The p66 subunit shows an overall structural similarity to the polymerase domain of *Escherichia coli* DNA Pol I and other polymerases. Without reverse transcriptase, the viral genome could not incorporate into the host cells, and therefore could not reproduce. This concept leads to the consideration of the pharmacological inhibitors of DNA polymerase for the treatment of cancer.

DNA polymerase enzyme is a vital enzyme for the regulation of multiple physiological cellular functions such as DNA repair, gene transcriptions, cell cycle progression, cell death, chromatin function, and genomic stability. Overconsumption of polymerase will result in cellular energetic depletion, mitochondrial dysfunction, and ultimately cellular necrosis (Abdelkarim et al., 2001; Aldinucci et al., 2007; Akiyama et al., 2001). This enzyme is essential for the process of viral replication and the production of a new virion which also leads us to considering it as a useful tool for the treatment of cancer.

Now the most successful available treatment today is reverse-transcriptase inhibitors [for example azidothymidine (AZT)] and protease inhibitors (Baeten, 2011; Thompson et al., 2010). These treatments interfere with enzymes that are needed for HIV to make copies of itself, a key step in the virus's attack on the cells of the immune system. CD4+ T-cells are one of our immune system components that are necessary to stimulate the activation of other immune cells that attack infectious particles (antigens) in the body. When these cells come under attack by HIV, the immune systems can no longer function effectively, and the body is incapable of combating the HIV.

Accordingly, the question that arises is why is our immune system not able to stop the consumption of the immune cells while the virus can? And why does the viral replication process have a continuous dynamic action with no signs of immune suppression? The answer depends on our vision of the HIV strategy. The HIV stimulates the cytotoxic CD8+ T-cells to act on CD4+ T-cell, the latter cell will try to inhibit this mechanism and such a phenomenon could be considered as a defensive mechanism but as long as this process continues and the inhibitors mechanism is gradually depleted, it will lead to a profound destruction of all CD4+ T-cell paving the way to the spread of the viral invasion. Therefore, we assume that the failure of immune cells to stop the viral replication mainly comes from the mystery stored in the functions of RT enzyme that makes our immune cells compatible

with its orders.

METHODOLOGY

Previous studies have proven that RT enzyme works like many other DNA polymerase, where the goal of HIV-1 RT enzyme is to convert the single stranded RNA genome into double-stranded DNA (Jaeger et al., 1998 ; Lenvin and Henry, 1997). Thus we assumed that introducing the reverse transcriptase and DNA polymerase enzyme in a combined form (available in experimental laboratories as Taq DNA polymerase, AMV Reverse Transcriptase, Promega Corporation Manufactured-date; 22, October, 2009, expiry date; 31 July, 2011) to the host subcutaneously will stimulate the immune system to produce neutralizing antibodies to directly inhibit these proteins that the virus is using for its cellular entry which will interfere ultimately with its replication process, considering this as a natural inhibitors to HIV and sparing patients infected with HIV from all serious side effects arising from conventional antiviral drugs available on the market.

Patient number 1

Mr Shady, is a 24 year old Egyptian male, HIV positive since February, 2009 with symptoms of early HIV infection such as joint pain, muscle ache, weakness and white spots on the tongue. Physical examination revealed hepatomegaly and splenomegaly, shallow and rapid respirations. Serological testing was positive for hepatitis A antibody, negative for hepatitis B core antibody and hepatitis C (HCV). CD4+ count were 270 cells/ μ l (normal range 350 to 550 cells/ μ l), viral load was 92,000 copies/ml, white blood cell (WBC) count 9,600 cells/ μ l, alanine aminotransferase (ALT) 67 IU/L (normal range 0 to 40 IU/L), aspartate aminotransferase (AST) 60 IU/L (normal range , 0 to 40 IU/L).

Patient number 2

Mr Mouhamed is a 26 year old Egyptian male diagnosed with HIV disease after he had developed a fever of unknown cause, abdominal pain, weight loss and sore throat. His CD4+ cell counts were 315 cells/ μ l, and a viral load of 105,000 copies/ml. WBC count were 10,800 cells/ μ l, negative antibodies to HCV, HBV, AST and ALT were within normal range.

Patient number 3

Mrs Aum ommr is a 34 year old female from Saudia Arabia with a history of HIV infection diagnosed in 2004 via sexual contact and history of pulmonary tuberculosis, presented with significant weight loss, chronic diarrhea, joint pain, muscle aches, chills, and lymphadenopathy. Serological testing showed CD4+ cell count of 180 cells/ μ l, viral load of 447,300 copies/ml , WBC count 4200 cells/ μ l, ALT 67 IU/L, and AST 42 IU/L. Serology for HCV and HBV were negative, and sputum for tuberculosis were positive in three different samples.

Patient number 4

Mrs Ayaa is a 23 year old HIV positive Egyptian female, presented immediately after she was diagnosed seeking medical treatment. Patient was asymptomatic. Serological testing showed CD4+ cell

count of 170 cells/ μ L, viral load of 4,300 copies, WBC count 5300 cells/ μ L, ALT and AST were within normal range, serology were negative for HCV, and positive for HBV and Hepatitis B surface antibody(HB S ab)

Patient number 5

Mr Ahmed a 38 year old HIV positive Egyptian male was diagnosed after returning to Cairo after spending most of his life abroad, the patient presented with right upper quadrant abdominal pain, nausea, anorexia, significant weight loss, weakness, and intermittent diarrhea. Serological testing showed CD4 count of 150 mm³, viral loads of 315,000 copies, WBC count 3400 cells/ μ L, negative serology for HCV and HBV, and normal liver enzymes.

Our hypothesis was tested on the patients after written informed consent was signed by each of them to participate in the trial, no significant side effects of the treatment were anticipated except for possible allergic reaction. The duration of the therapy was 24 weeks, every patient was trained to self inject 0.10 cc of the combination (stored in 3 cc vials) twice daily after breakfast and after dinner as a total of 120 injection (total of 4 vials). Patients were followed up weekly in the outpatient clinic for observation only.

RESULTS

After 24 weeks of the beginning of the therapy, the immunological testing of the above patients was repeated, showing surprisingly undetectable viremia (reference range < 16 copies/ml) for all of the patients with significant elevation in CD4+ T-lymphocytes above 500 cells/ml. The most important finding of great immunological value is that HIV antibodies by enzyme linked immunosorbent assay (ELISA) testing were negative also, the patients reported significant improvement of their physical activities and the constitutional symptoms of HIV including malaise, fatigue, weight loss and joint pain.

DISCUSSION

This study introduced a novel biological enzymatic mixture comprising reverse transcriptase and DNA polymerase enzyme (VK 25 RD) for the treatment of HIV/AIDS differing from all remedial methods which depend on treating the viral infection by immune inhibitors for the virus replicating materials reverse transcriptase, DNA. The recent trend of treating HIV/AIDS is to combine at least three drugs from two different classes. These classes include: Nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), fusion inhibitors and integrase inhibitor. They generally work by disabling the formation of proteins needed by the virus to copy itself (NNRTIs, PIs and integrase inhibitors) or blocks the HIVs entry into the CD4 cells (fusion inhibitors). The side effects of these

drugs are remarkable, their use, whatever the time it takes never leads to complete cure, but it aims to ameliorate the clinical picture, to increase the CD4 cell count and decrease the viral load. Versus the strategy of these drugs which aims to block HIV copying by direct interference through the drug action, our hypothesis aims to enhance the immune system of the patient to build its own neutralizing antibodies against the virus.

This study and its promising results could be a step towards a real solution for the global problem. However, we have to confess that there was a limitation concerning our study due to the small sample size which emerged from the limited infected cases of HIV/AIDS within reach in Cairo. Besides, a restricted budget was available which also challenged us, so we emphasize that a further extended and tedious study is needed to evaluate the benefits and values of the compound.

Conclusion

From the results we found that stimulating the body with this combination therapy produced "broadly neutralizing antibodies" that will be the key for further research to explore the possibilities of developing a prophylaxis (vaccine) and/or curative treatment for HIV infection.

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