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HIV Report

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INTRODUCTION

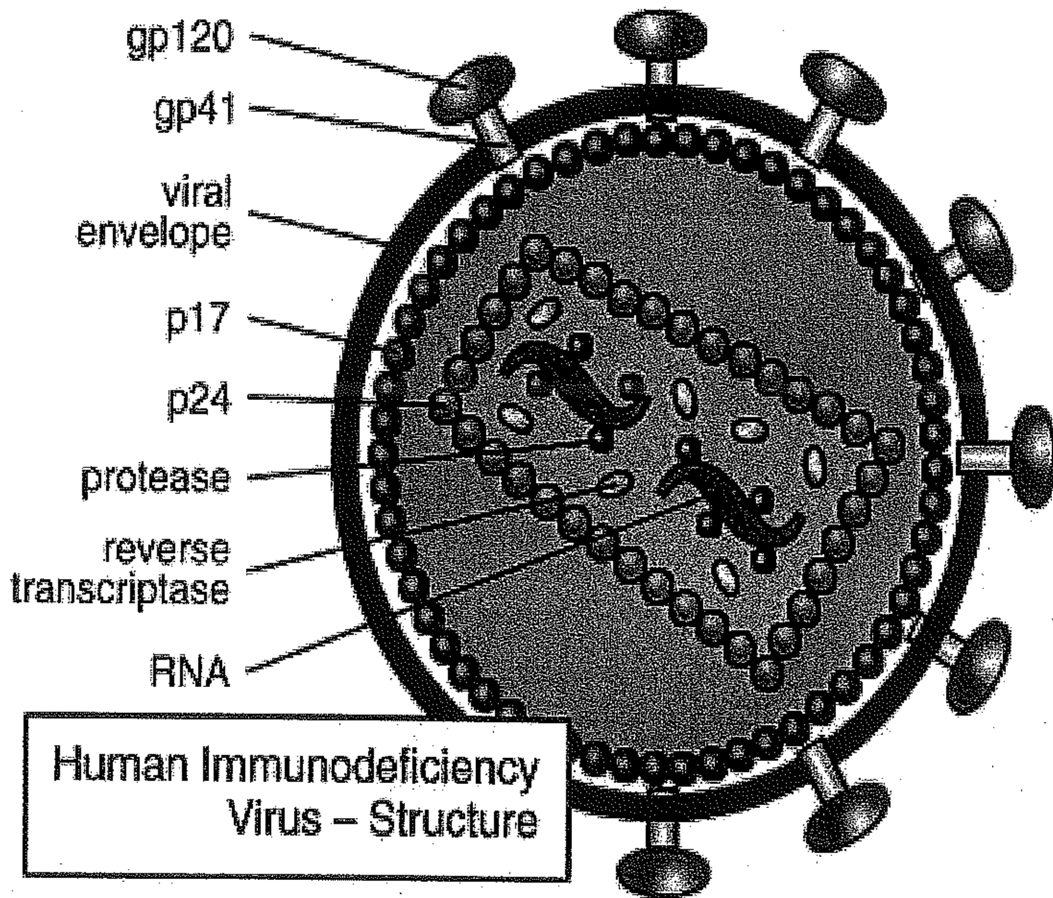
Following up on the impressive results of ASAP 10 & 22 ppm on the HBV Reverse Transcriptase and HBV DNA polymerase it was thought worthwhile to test these solutions on HIV too.

Most of the anti retro viral drugs that target HIV replication have the problem of cytoxicity as well as rapid resistance development. This leads one to search for safer and effective anti retroviral drugs.

With the above in mind it was decided to see if ASAP solution has antiretrovial activity by carrying out

1. Infectivity Inhibition assay
2. P^{24} linked Immonosorbent assay
3. Reverse Transcriptase Inhibition Assay.

HIV VIRUS



Human Immunodeficiency Virus – Structure

LIFE CYCLE OF HIV

HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell. CD4 is present on the surface of many lymphocytes, which are a critical part of the body's immune system. Recent evidence indicates that a coreceptor is needed for HIV to enter the cell. This recognition of HIV coreceptors and progress in understanding how HIV fuses with the cell has opened up new possibilities for antiviral drugs. A number of new agents are being designed to prevent infection by blocking fusion of HIV with its host cell.

Following fusion of the virus with the host cell, HIV enters the cell. The genetic material of the virus, which is RNA, is released and undergoes reverse transcription into DNA. An enzyme in HIV called reverse transcriptase is necessary to catalyze this conversion of viral RNA into DNA. Inhibitors of reverse transcriptase, such as AZT, were the first anti-HIV medications, and are still a critical part of treating patients who have HIV. Reverse transcriptase inhibitors are divided into two classes-nucleoside analogues and non-nucleoside reverse transcriptase inhibitors-based on their structure and how they inhibit reverse transcriptase.

Once the genetic material of HIV has been changed into DNA, this viral DNA enters the host cell nucleus where it can be integrated into the genetic material of the cell. The enzyme integrase catalyzes this process, and inhibitors of integrase are under study as a new way to block HIV replication. Once the viral DNA is integrated into the genetic material of the host, it is possible that HIV may persist in a latent state for many years. This ability of HIV to persist in certain latently infected cells is the major barrier to eradication or cure of HIV. For this reason, based on our current knowledge, patients must remain on anti-viral therapy for life.

Activation of the host cells results in the transcription of viral DNA into messenger RNA (mRNA), which is then translated into viral proteins. The new viral RNA forms the genetic material of the next generation of viruses. The viral RNA and viral proteins assemble at the cell membrane into a new virus. Amongst the viral proteins is HIV protease, which is required to process other HIV proteins into their functional forms. Protease inhibitors, one of the most potent types of anti-viral medications, act by

blocking this critical maturation step. Following assembly at the cell surface, the virus then buds forth from the cell and is released to infect another cell.

Unless the HIV lifecycle is interrupted by treatment, the virus infection spreads throughout the body and results in the destruction of the body's immune system. With current anti-viral medications, such as reverse transcriptase inhibitors and protease inhibitors, HIV infection can be contained. However, a great deal more must be achieved before AIDS epidemic is brought under control. One important immediate goal is to design new, more potent medications that are easier to take and have fewer side effects. However, the ultimate challenges are to use our understanding of the HIV lifecycle to develop medications that will eradicate HIV from people who are already infected and to create a vaccine that will prevent new infections in the future.

INFECTIVITY INHIBITION ASSAY (KARN 1995)

Objective

To measure infectivity of the virus stock , and check if ASAP solution inhibits the same.

Princple

The assay involves the development of large multinucleated cells(Synctium) that may arise in infected cultures following fusion of HIV infected cells with uninfected CD4 positive cells. The fusion reaction is mediated by gp41,which is exposed on the surface of virus particles or infected cells following the interaction of CD4 and the extra cellular HIV envelop glycoprotein, gp120.

Equipment

Incubator .

Material

1. Synthetic tissue culture medium
2. H9 cells
3. Virus stocks
4. Test samples (ASAP 10 ppm & ASAP 22 ppm)

Procedure

1. Culture H9 cells in synthetic tissue culture medium to obtain appropriate cell density.
2. Incubate H9 lymphocytes for 1 hr. with virus in presence and in absence of the test compound.
3. Wash the cells thoroughly and resuspend in the culture medium.
4. After 3 days of incubation, perform p 24 antigen capture assay to check level of infectivity in presence of test compound and in the absence of the same.
5. Microscopically check for synctia formation

Results

Sr.	Test Samples	% Infectivity Inhibition
1.	ASAP (10 ppm)	Nil
2.	ASAP (22 ppm)	Nil
3.	Ctrl- PBS (100 µl)	Nil

There was no decrease in p24 antigen antigen formation whichwas similar to the PBS control indicating that the ASAP solution was not capable of reducing infection.

Similarly Synctia (multinucleated giant cells) were seen in all 3 samples indicating infectivity was not curtailed by ASAP.

P24 ENZYME-LINKED IMMUNOSORBENT ASSAY (KARN 1995)

Objective

To measure effect of ASAP solutions (10 ppm , 22 ppm) on HIV core antigen p24. production

Principle

Viral capsid protein p24 is captured by an antibody bound to the surface of a microwell & this bound antigen is detected following successive incubations with a biotinylated secondary antibody & conjugated streptavidin- horse –radish peroxidase.

The subsequent addition of an appropriate substrate such as tramethylbenzidine results in the development of colour which is read at 450 nm in a microtitre plate reader.

The intensity of colour is directly proportional to the amount of p24 in pg/ ml to ng /ml .

Equipment

Incubator .

Material

1. Synthetic tissue culture medium
2. H9 cells
3. Virus stocks
4. Test samples (ASAP 10 ppm & ASAP 22 ppm)

Procedure

1. Culture H9 cells in tissue culture medium to obtain appropriate cell density.
2. Inoculate the virus in the cells with known cell density.
3. After appropriate incubation*, add the test compound to the cultured cells.
4. Test the culture supernatants for the p24 antigen using the standard ELISA test (commercially available p24 antigen capture assay kit).

Results

Sr. No.	Test Samples	% p24 Secretion Inhibition
1.	ASAP (10 ppm) 100 µl	Nil
2.	ASAP (22 ppm) 100 µl	16.67
3.	Ctrl- PBS 100 µl	Nil

REVERSE TRANSCRIPTASE ASSAY

Objective

To detect percentage inhibition of reverse transcriptase by ASAP solution using liquid scintillation.

Principle :

Viral extracts are incubated with radiolabelled nucleotides and an active inhibitor. Percent inhibition is calculated based on amount of viral nucleic acid synthesized with respect to positive & negative controls.

Equipment :

1. Liquid Scintillation Counter (Blue Star)
2. Incubator .

Material :

1. Micropipette
2. Sterile micropipettar tips
3. Ionic paper (DEAE)
4. Sterile eppendoff tubes
5. Commercial Virus Preparation of Moloney Murine Leukaemia Virus RT (MoMuLV)
6. Test Sample (ASAP 10 ppm & ASAP 22 ppm)
7. EDTA
8. Trichloroacetic acid (TCA)

Procedure :

1. 25µl mixture of virus extract and radiolabelled nucleotide was prepared.
2. To this reaction mixture 3µl of active inhibitor was added and mixture was incubated at 37°C for 2 hrs.

3. Reaction was stopped by adding EDTA.
4. Then it is filtered through ionic paper.
5. Paper was washed for 2-3 times dried and then checked for radioactivity on liquid scintillation counter.

Results :

Sr. No.	Test Samples	% Reverse Transcriptase Inhibition
1.	ASAP (10 ppm) 100 µl	30.19
2.	ASAP (22 ppm) 100 µl	37.74
3.	Ctrl- PBS 100 µl	Nil
4.	Ctrl- AZT 0.625 µg/ml	16.0

CONCLUSION & INTERPRETATION

Both ASAP 10 ppm & 22 ppm show dose related inhibition of HIV viral Reverse Transcriptase activity which is significantly more than the positive control AZT.

p24, the HIV core antigen, secretion was inhibited only by 22 ppm & not 10 ppm . This result taken along with the RT inhibition demonstrates that higher concentrations of ASAP are able to inhibit viral replication.

However, infectivity i.e. the capacity of the virus to infect CD4 cells is not inhibited by ASAP solution since presence of large multinucleated cells(Synctia) were seen in the medium.

This indicates fusion of HIV infected cells with uninfected cells through the mediation of gp41 & gp120, even in the presence of 100 µl ASAP solution (10 ppm & 22 ppm). Further higher concentrations of ASAP solution would possibly give more encouraging results.

References :

J. Karn (1995) : HIV volume 1 & 2
A Practical Approach
IRL Press, Oxford University

APPENDIX

APPENDIX – I

Media Composition :

1. Synthetic Tissue Culture Medium

Material :

1. Tissue culture flasks
2. Round bottom 96- well microtitre plates
3. Polypropylene sterile 15 ml tubes
4. RPMI 1640 medium with 10% fetal bovine serum (RPMI- 10)
5. 10 mg/ml polybrene
6. RPMI-10 with 2 μg/ml of polybrene (RPMI-10/2)

Procedure :

1. Centrifuge H9 cells in 15 ml conical tubes (1000 g for 5 minutes at RT).
2. Resuspend cells in RPMI-10/2.
3. Add 2 X 10^6 cells per 15 ml conical tube and centrifuge at 1000 g for 5 min.
4. Resuspend cells in 1 ml of RPMI-10/2 containing virus (5 X 10^4 to 5X 10^6 ^{32}P c.p.m. RT activity)
5. Incubate cells for 2 hrs. at 37^0 C, mixing every 15-30 min.
6. Add 2 ml of RPMI-10/2 and transfer to T25 flasks;the virus is not removed.
7. Add 3 ml of RPMI-10/2 the next day; the total volume of the medium is now 6 ml.
8. Feed the cells every 2 days by removing 4 ml of medium without disturbing the cells. Maintain the cell density.

2. RT Assay Mixture :

50 mM Tris-HCl pH 7.8, 75 Mm KCl, 2 Mm DTT, 5Mm Mg Cl_2, 5 μg/ml, poly (A), c. 1.6 μg/ml oligo (dT) 12-18, 0.05 % NP-40, 1Mm EDTA, 10 μCi/ml (α-^{32}P) dTTP.