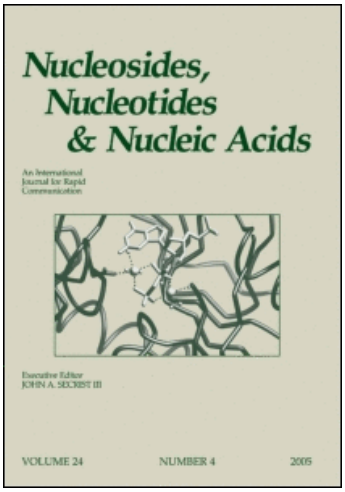

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Inhibition of Human Immunodeficiency Virus Type-1 (HIV-1) Replication by Immunor (Im^{28}), a New Analog of Dehydroepiandrosterone

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INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) REPLICATION BY IMMUNOR (IM^{28}), A NEW ANALOG OF DEHYDROEPIANDROSTERONE

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ABSTRACT:

The inhibition of HIV-1 replication *in vitro* by Immunor 28 (IM^{28}), an analog of dehydroepiandrosterone (DHEA), was monitored using the HIV-1 laboratory wild-type strain IIIB. Evaluation of the 50% inhibitory dose (IC_{50}) revealed a decrease in HIV-1 replication giving an IC_{50} value around 22 μM . The toxicity of the drug has been determined also, in MT2 cells and PBMCs. 60 μM of IM^{28} provoked a 50% decrease in cell viability while DHEA caused the same decrease at 75 μM in MT2 cells. These values are 125 μM for IM^{28} in PBMCs and 135 μM for DHEA. Thus, DHEA is less toxic than IM^{28}, but IM^{28} has a higher antiviral activity.

This paper is dedicated to the memory of Professor A. Krayevsky.

INTRODUCTION

DHEA, one of the highly concentrated adrenal hormones in humans, has been associated with a broad range of beneficial biological activities, including a suppressive effect against HIV-1[7]. IM^{28}, that was evaluated in this study, is a recently developed analog of DHEA. In HIV-1 infected patients treated in Gabon, IM^{28} was shown to decrease plasma viral load by 62.29% in about four months, and to stabilize or increase patient weights[1]. IM^{28} was also well tolerated by patients, showing very few side effects[1]. It was also demonstrated in clinical trials that IM^{28} increased the numbers of T lymphocytes in HIV-1 infected patients, a sign of reactivation of the immune system[1]. IM^{28} makes the cell wall less permeable to virus entry, possibly helping to prevent infection of new cells[1]. During the same clinical trial, important effects on opportunistic infections were observed. In combination with other antiviral drugs, IM^{28} showed a synergistic action in the treatment of AIDS. In vitro experiments showed that the drug inhibited HIV-1 envelope glycoprotein-mediated cell fusion[2].

MATERIAL AND METHODS

Viruses and cells. The infectious laboratory wild-type strain IIIB was utilized to monitor inhibition of virus replication. Clinical HIV-1 isolates were obtained by co-culturing peripheral blood mononuclear cells (PBMC) from patients with cord blood mononuclear cells (CBMC) as previously described[3].

In vitro antiviral activity of IM^{28}. Then, by using *in vitro* activity of IM^{28}, we first determined the 50% inhibitory dose (IC_{50}) by growing the IIIB strain in the presence of increasing drug concentrations in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 2 μM L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. Then, syncytium-inducing (SI) and reverse transcriptase (RT) assays were used to determine IC_{50} values as described[3]. CBMCs were used for these experiments.

Determination of cytotoxicity. Cytotoxicity was determined by growing the IIIB strain under the same conditions as for IC_{50} determinations, and by using day 3 or 4 infection cell numbers to establish the concentration of drug that was toxic for 50% of the cells ($CCID_{50}$). Both CBMCs and the MT2 Tcells line were used.

Syncytium induction assay. MT2 cells were pre-incubated with appropriate concentrations of drugs for one hour at 37°C in 5% CO_2 incubator and subsequently infected with the HIV-1 IIIB strain at 50% tissue infective dose ($TCID_{50}$) of 0.1. After two hours, the cells were washed and maintained in tissue culture medium at the same drug concentration used during both pre-incubation and infection. Cells were fed after 3 days with culture medium containing an appropriate concentration of drug, and HIV-1 induced cytopathic effect (CPE) was monitored for 8 days. The reverse transcriptase (RT) assay was carried out in duplicate using cell-free culture supernatant as previously described[3].

RESULTS

The *in vitro* activity of IM^{28} was measured by determining the dose needed for 50% inhibition of wild type virus replication (IC_{50}) in MT2 cells (Table 1). We also measured the IC_{50} value of DHEA, and AZT. In the syncytium induction assay, 2% phosphate buffered saline (PBS) in culture medium was used as a control. DHEA and IM^{28} were solubilized in ethanol 95% prior to resuspension in culture medium. The results showed an IC_{50} value around 22 μM for IM^{28} and 50 μM for DHEA. The $CCID_{50}$ was 75 μM for DHEA with 50 % inhibition of cell growth and 60 μM for IM^{28} in MT2 cells (Table 2). In PBMCs, the toxicity was 135 μM for DHEA and 125 μM for IM^{28} (Table 2). We also monitored inhibition of virus replication over 8 days by using RT activity assay and CPE inducing assay. The results showed a time-dependent decrease in viral growth (Figure 1 and 2).

DISCUSSION

In this study, we have investigated the *in vitro* antiviral activity of IM^{28}. IC_{50}, and

TABLE 1 . IC_{50} Values of HIV-1 isolates for various drugs in MT2 cells.

Virus	IC50 (μM)		
	AZT	DHEA	IM^{28}
Wild-type IIIB	0.001	50	22
Wild-type isolate 4246	0.001	50	22

Results were determined on the basis of RT activity in culture fluids as described.

TABLE 2. $CCID_{50}$ values for IM^{28} and DHEA in MT2 cells and PBMCs

Drug	$CCID_{50}$ Values (μM) in MT2	$CCID_{50}$ Values (μM) in PBMCs
DHEA	75	135
IM^{28}	60	125

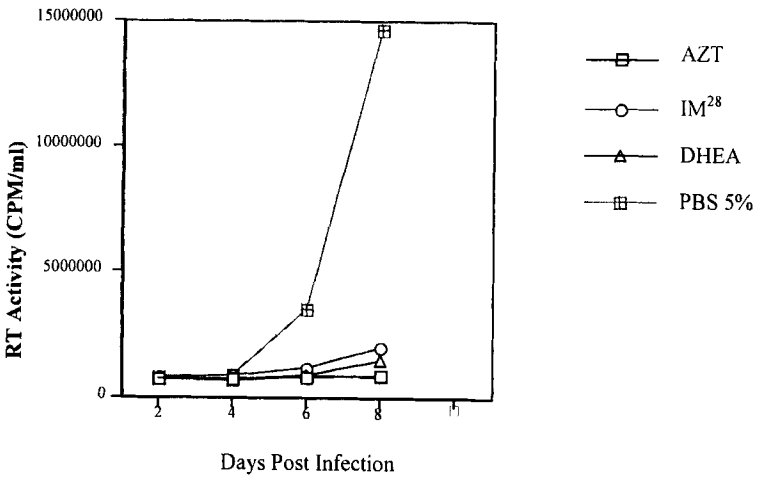

FIG.1 Sensitivity curve of IIIB to AZT, DHEA and IM^{28}.

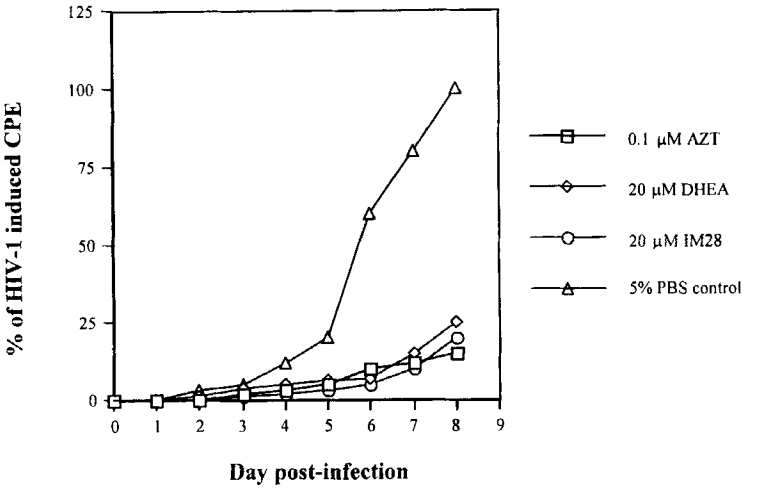

Figure 2: HIV-1 inhibition by IM28, DHEA and AZT

$CCID_{50}$ values obtained from assays in CBMC were generally consistent with those previously obtained with DHEA of which IM^{28} is an analog. These results are consistent with *in vivo* results obtained during a clinical trial in 1999 in Gabon[1,6]. IM^{28} displayed more toxicity than DHEA in MT2 cells, and both products showed low toxicity in PBMCs, consistent with previous studies showing the anti-cancer cell activity of DHEA[4,5]. These results demonstrated that the antiviral activities of DHEA and IM^{28} were more efficient after day six of infection and that IM28 is more active against HIV-1 than DHEA. DHEA and its sulfate derivative have been reported to inhibit both RNA and DNA viral expression, including that of HIV-1[2]. Our results are consistent with these findings. Thus, the antiviral activity of both IM^{28} and DHEA suggest that these drugs could be investigated as potential therapeutic agents for HIV-1 disease.

Further studies are needed to determine whether IM^{28} and DHEA will be active against drug-resistant strains of HIV-1.

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