INHIBITION OF INFECTIVITY OF HUMAN IMMUNODEFICIENCY VIRUS BY OXETANOCIN

Sir:

Human immunodeficiency virus (HIV) or human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) is a causative agent of acquired immune deficiency syndrome (AIDS)[1,2]. During screening substances that will inhibit infection of human T-cell lines with HIV, we found that 9-[(2*R*,3*R*,4*S*)-3,4-bis(hydroxymethyl)-2-oxetanyl]adenine, namely oxetanocin[3], had the antiviral effects.

Antiviral effects of oxetanocin were examined using MT-4 and ATL-1K cells[4,5] and two strains of HIVs, HTLV-III$_B$[6] and HIV[GUN-1][7]. MT-4 cells were derived from human T-cells and producing human T-cell leukemia virus type 1 persistently. MT-4 cells were reported to be very sensitive to HIV[8]. These cells were maintained in RPMI 1640 culture medium containing 10% heat-inactivated fetal calf serum. HTLV-III$_B$ and HIV[GUN-1] were prepared from human T-cell lines infected with them as described elsewhere[7].

At first, effects of oxetanocin on growth of MT-4 and ATL-1K cells were examined (Fig. 1).

Fig. 1. Effects of oxetanocin on growth of MT-4 cells.

MT-4 cells were cultivated in the presence of oxetanocin at concentrations of 30 (●), 10 (▲), 3 (□), 1 (△) and 0 (○) μg/ml.

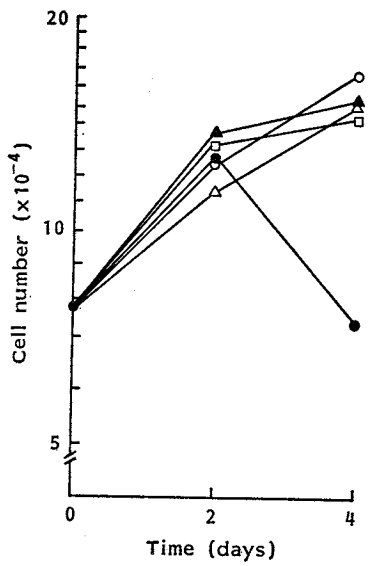
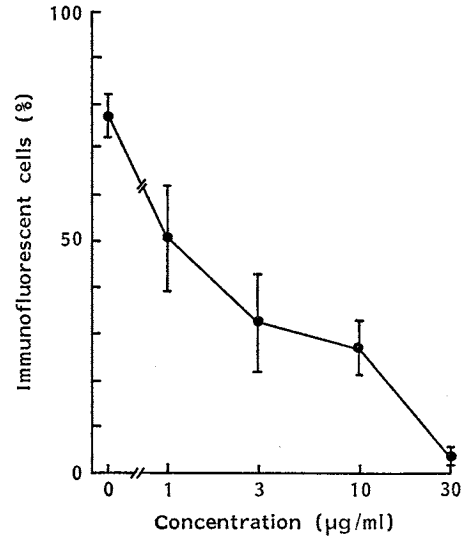


Fig. 2. Effects of oxetanocin on expression of viral antigens after infection with HIV.

Percentages (mean±SD) of viral antigen-positive cells by indirect immunofluorescence assays are shown.



MT-4 cells were seeded into 24-well plates at 8×10^4 cells/ml in the presence of the indicated concentrations of oxetanocin. Stock solution of oxetanocin contained 10 mg/ml in dimethyl sulfoxide. Cell numbers were counted by a hemocytometer on days 2 and 4. Oxetanocin was not inhibitory to cell growth during the first 2 days. No inhibition of cell growth was observed after another 2 days cultivation at concentrations of up to 10 μg/ml. The growth was inhibited at a concentration of 30 μg/ml. Growth of ATL-1K cells was not inhibited even in medium containing 30 μg/ml of oxetanocin (data not shown).

Effects of oxetanocin on infection with HIV were examined as follows: MT-4 cells were seeded into 24-well plates at 1×10^5 cells/ml well. Then 100 μl of oxetanocin diluted with phosphate buffered saline was added in duplicate. Five hours later, $10^3\sim10^4$ plaque-forming units of HTLV-III$_B$ or HIV[GUN-1] were added to each well. The plates were incubated for 4 days at 37°C under 5% CO_2. Then aliquots were taken from each well in duplicate. After centrifugation, cells were smeared onto slide glasses, fixed with acetone and examined by indirect immunofluorescence assay as described elsewhere[7]. Briefly, cells were treated with serum of a hemophiliac or AIDS patient, positive for

antibody against HIV. Then cells were reacted with fluorescent isothiocyanate-conjugated rabbit IgG against human IgG and examined by a fluorescence microscope. Fig. 2 shows that oxetanocin inhibited expression of HIV antigens markedly. Figs. 1 and 2 indicated that oxetanocin inhibited infection with HIV at concentrations not inhibitory to growth of MT-4 cells. Inhibition of HIV infectivity was also observed when MT-4 or ATL-3I cells were infected with HIV[GUN-1] or HTLV-III_B in the presence of oxetanocin (data not shown). Although exact mechanisms of inhibition of HIV infectivity by oxetanocin are not clear yet, it is possible that oxetanocin inhibits reverse transcription of HIV genome after it is phosphorylated in the cells like 3′-azido-3′-thymidine or 2′,3′-dideoxynucleosides[9]. It remains to be determined whether oxetanocin can be used as a therapeutic agent for AIDS.

Acknowledgment

We thank Drs. R. C. GALLO and I. MIYOSHI for providing HTLV-III_B virus and MT-4 cells, respectively.

HIROO HOSHINO
NOBUAKI SHIMIZU

Department of Hygiene, Gunma University, School of Medicine, 3-39-22 Maebashi, Gunma 371, Japan

NOBUYOSHI SHIMADA
TOMOHISA TAKITA

Research Laboratories, Pharmaceutical Group, Nippon Kayaku Co., Ltd., 3-31-12 Shimo, Kita-ku, Tokyo 115, Japan

TOMIO TAKEUCHI

Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received April 30, 1987)

References

1) BARRÉ-SINOUSSI, F.; J. C. CHERMANN, F. REY, M. T. NUGEYRE, S. CHAMARET, J. GRUEST, C. DAUGUET, C. AXLER-BLIN, F. VÉZINET-BRUN, C. ROUZIOUX, W. ROZENBAUM & L. MONTAGNIER: Isolation of T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220: 868~871, 1983
2) GALLO, R. C.; S. Z. SALAHUDDIN, M. POPOVIC, G. M. SHEARER, M. KAPLAN, B. F. HAYNES, T. J. PALKER, R. REDFIELD, J. OLESKE, B. SAFAI, G. WHITE, P. FOSTER & P. D. MARKHAM: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224: 500~503, 1984
3) SHIMADA, N.; S. HASEGAWA, T. HARADA, T. TOMISAWA, A. FUJII & T. TAKITA: Oxetanocin, a novel nucleoside from bacteria. J. Antibiotics 39: 1623~1625, 1986
4) MIYOSHI, I.; H. TAGUCHI, I. KUBONISHI, T. YOSHIMOTO, Y. OHTSUKA, Y. SHIRAISHI & T. AKAGI: Type C virus-producing cell lines derived from adult T-cell leukemia. Gann Monogr. 28: 219~228, 1982
5) HOSHINO, H.; H. ESUMI, M. MIWA, M. SHIMOYAMA, K. MINATO, K. TOBINAI, M. HIROSE, S. WATANABE, M. ICHIMARU & T. SUGIMURA: Establishment and characterization of 10 cell lines derived from patients with adult T-cell leukemia. Proc. Natl. Acad. Sci. U.S.A. 80: 6061~6065, 1983
6) POPOVIC, M.; M. G. SARNGADHARAN, E. READ & R. C. GALLO: Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224: 497~500, 1984
7) TAKEUCHI, Y.; M. INAGAKI, N. KOBAYASHI & H. HOSHINO: Isolation of human immunodeficiency virus from a Japanese hemophilia B patient with AIDS. Jpn. J. Cancer Res. 78: 11~15, 1987
8) HARADA, S.; Y. KOYANAGI & N. YAMAMOTO: Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science 229: 563~566, 1985
9) MITSUYA, H. & S. BRODER: Strategies for antiviral therapy in AIDS. Nature 325: 773~778, 1987