# KIJIMICIN: AN INHIBITOR OF HUMAN IMMUNODEFICIENCY VIRUS IN ACUTELY AND CHRONICALLY INFECTED CELLS

Sir:

A new polyether antibiotic, kijimicin (Fig. 1) has recently been identified in the culture filtrate of *Actinomadura* sp. MI215-NF3[1]. This agent exhibits high anticoccidial activity. In the present communication, we report its anti human immunodeficiency virus (HIV) activity in both primary and chronic infection.

The experiment analyzing effects on primary infection was performed according to the following. H9 cells[2] were pretreated with serially diluted kijimicin at 37°C for 30 minutes and then infected with HIV at a multiplicity of 0.05. Cells were incubated at a density of $2.5 \times 10^6$/ml for 90 minutes at 37°C to permit adsorption of viral particles and then diluted with fresh media 1 : 10 for culturing in a 96-well plate. On day 4, the cells were split 1 : 5 and on day 7 the culture fluid was harvested for reverse transcriptase (RT) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays.

The RT assay[3] was performed to estimate the concentration of viral particles in the culture supernatant. In brief, 10 $\mu$l of supernatant containing HIV particles was disrupted with 10 $\mu$l of detergent solution (50 mM Tris-HCl pH 8.0, 10 mM dithiothreitol (DTT), 300 mM KCl, 0.5% Triton X-100) in a 96-well round bottom plate. Following 15 minutes incubation at 4°C add 25 $\mu$l of RT buffer containing 50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 5 mM DTT, 0.25 U/ml poly(rA)oligo(dT)$_{12\sim18}$, 15 $\mu$Ci/ml [$^3$H]dTTP. The reaction mixtures were incubated at 37°C for 4 hours and 15 $\mu$l of mixture was then spotted onto DE81 Whatman filter paper, dried, washed with 2 × SSC, and rince with ethanol. Radioactivities of these filters were counted by a Beckman scintillation counter. The MTT assay[4] was used to measure the number of living cells as follows: Take 50 $\mu$l of cell suspension and transfer to a new 96-well flat bottom plate containing 50 $\mu$l of incomplete RPMI medium. Add 10 $\mu$l of 5 mg/ml MTT-phosphate-buffered saline (PBS) and incubate at 37°C for 4 hours. Then add 100 $\mu$l of 0.04 N HCl-2-propanol and mix thoroughly by repeated pipetting. Measure absorbance on a ELISA plate reader with a test wavelength of 550 nm and reference wavelength of 630 nm.

As shown in Fig. 2, kijimicin treatment resulted in a concentration dependent inhibition of HIV replication in this acute primary assay system. HIV production was completely abolished at concentrations higher than 10 $\mu$g/ml, and $IC_{50}$ was observed at 5.0 $\mu$g/ml that produced no cytotoxicity. The $IC_{50}$ of MTT was at 150 $\mu$g/ml, consequently the ratio of $IC_{50}$ values for MTT to RT was 30. Identically treated uninfected cultures studied in parallel showed no change in cellular viability as compared to infected cultures (data not shown).

To exclude the possibility that kijimicin directly inhibited reverse transcriptase in the RT assay, we used an immunofluorescence assay[5] to study expression of HIV antigen. No fluorescence was detectable when using kijimicin at 10 $\mu$g/ml. Moreover, at this concentration, no change in viability was seen in the kijimicin treated cultures and RT activity was nearly background. These results suggest that kijimicin inhibits HIV replication in the absence of cytotoxicity over a wide range.

We next examined the antiviral activity of kijimicin in chronically HIV-infected U937 cells. These cells ($2.5 \times 10^5$/ml) were seeded in a 96-well plate in the presence of (10 $\mu$l) serial dilutions of kijimicin. Culture supernatant was harvested at 24, 48, 72, 96 hours for RT assay and at 96 hours, the MTT assay was performed to examine cytotoxicity. The results demonstrate that kijimicin also has anti HIV activity in chronically infected cells (Fig. 3). While the $IC_{50}$ ratio is less than that obtained in acute infection, the higher cytotoxicity may have resulted from adding the agent every 24 hours to assure continuous exposure in the absence of knowing the half life of kijimicin.

In order to define the inhibitory mechanism of

Fig. 1. Structure of kijimicin.

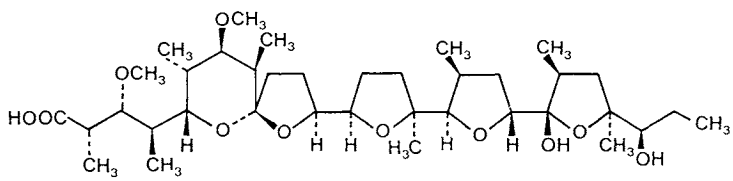


Fig. 2. The inhibitory effect on acute infection.

% of inhibition of RT is shown by ●, % of inhibition of MTT is shown by ○.

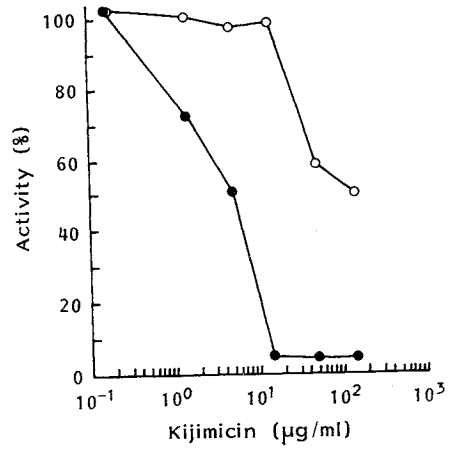
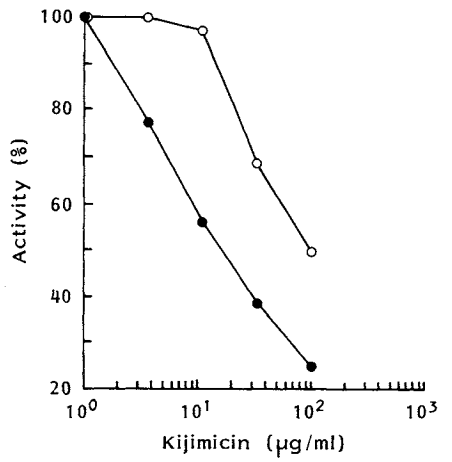



Fig. 3. The inhibitory effect on chronic infection.

% of inhibition of RT is shown by ●, % of inhibition of MTT is shown by ○.




kijimicin, we next examined the influence of various treatment periods with this agent. Complete inhibition of viral replication was obtained when kijimicin was added to a mixture of H9 cells already incubated with HIV-1 for 2 hours. Furthermore, in a marked contrast with dextran sulfate, pretreatment of cells with kijimicin had no effect on subsequent infection with HIV-1 (data not shown). In preliminary studies of primary infection, we have found accumulation of HIV-1 viral particles on the surface of kijimicin treated cells. These findings suggested that kijimicin may act on the internalization step in HIV replication. Indeed, monensin, another polyether, is known to inhibit the entry process of picornaviruses by increasing intravesicular pH[6]. Moreover, MADDON *et al.* have indicated that HIV entry by CD4 receptor mechanisms requires a low pH environment of the endosome[7]. In contrast, kijimicin was also effective against chronically infected cells, and monensin was also a potent inhibitor of the endoproteolytic cleavage of gp160[8]. Therefore, kijimicin may have other inhibitory mechanisms.

In summary, kijimicin is a promising agent for treating HIV infection and further studies are warranted in investigating the use of this agent against HIV infection.

Acknowledgments

We would like to thank SATOE OHKI for her excellent secretarial assistance and MORITO SAKAUE for technical assistance. This research was supported by Grant-in-Aid from the Ministry of Education, Science and Culture.

MARIKO NAKAMURA
TSUNEYA OHNO*

The Jikei University School of Medicine,
3-25-8 Nishi-shinbashi, Minato-ku,
Tokyo 105, Japan

SETSUKO KUNIMOTO
HIROSHI NAGANAWA
TOMIO TAKEUCHI

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

(Received November 27, 1990)

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