Suppression of HIV-1 replication in peripheral blood mononuclear cells by fasudil

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Abstract : Fasudil is a potent inhibitor for various protein kinases such as myosin light chain kinase and protein kinase C. It has been used as a drug for improvement of intracranial vasospasm and following ischaemic diseases. In this report, we demonstrate that fasudil suppressed the replication of human immunodeficiency virus type 1 (HIV-1) in mitogen-activated peripheral blood mononuclear cells. Our finding shows that fasudil may be useful as a new and distinct chemotherapeutic agent against HIV-1 infection. J. Med. Invest. 44 : 211-214, 1998

Key Words : *fasudil, protein kinase inhibitor, HIV-1*

INTRODUCTION

Fasudil (1-(5-isoquinolinesulphonyl)-homopiperazine) is a newly developed drug which improves intracranial ischemia as a result of the dilating effect of vasospasm (1, 2). The neuroprotective property of fasudil is due to its effect that inhibits the activities of various protein kinases including myosin light chain kinase, cyclic AMP-dependent protein kinase, cyclic GMP-dependent protein kinase and protein kinase C (3, 4).

Previous studies have shown that H-7, a protein kinase inhibitor, inhibits the activation of transcription of human immunodeficiency virus type1 (HIV-1) long terminal repeat (5) and HIV-1 replication in chronically infected cell lines (6). These effects of H-7 are caused by blockage of the protein kinase C-dependent cascade (5, 6). The similar efficacy of H-7 and fasudil as kinase inhibitors led to an idea that fasudil inhibits HIV-1 replication. If so, fasudil would be a candidate as a new anti-HIV-1 agent whose mechanism is different from that of currently available anti-HIV-1 drugs targeted for viral reverse transcriptase (RT) and protease. To address this issue, we examined the potential suppressive effect of fasudil on the replication of HIV-1 *in vitro*.

MATERIALS AND METHODS

Cells

A human cervical cell line, HeLa, was maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum. CD4-positive human lymphocytic cell lines, CEMx174, Jurkat, M8166, and MT-4, were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. Human peripheral blood mononuclear cells (PBMCs) were prepared and used as previously described (7).

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Cell proliferation assay

Cell proliferation was evaluated by the modified MTT method designated WST-1 as indicated in the manufacture's instruction (Dojindo Laboratories, Kumamoto, Japan).

Transfection and infection

For transfection, uncleaved plasmid DNA designated pNL432 (an infectious molecular clone of HIV-1) (8) was introduced into HeLa cells by the calcium-phosphate coprecipitation method (8). CD4-positive cell lines and PBMCs were infected with cell-free virus samples prepared from transfected HeLa cells as previously described (9).

RT assay

Virion-associated RT activity was measured as described previously (10).

RESULTS

Cytotoxicity of fasudil for T cell lines and PBMCs

As a first step to monitor the effectiveness of fasudil (Asahi Chemical Industry Co., Ltd., Shizuoka, Japan) against HIV-1, its cytotoxicity for various cell types was evaluated (Fig.1). In a human $CD4^+$ leukemia cell line, Jurkat, obvious cytotoxic effects were observed when fasudil was added to the culture at concentrations higher than 100μM, and no cytotoxicity was detected at ~37.5 μM of fasudil (Fig.1 A). Similar results were obtained in the other human T cell lines, CEMx174, M8166, and MT-4 (data not shown). In phytohemagglutinin P (PHA-P) (Difco Laboratories, Detroit, USA.)-stimulated PBMCs, the cytotoxic

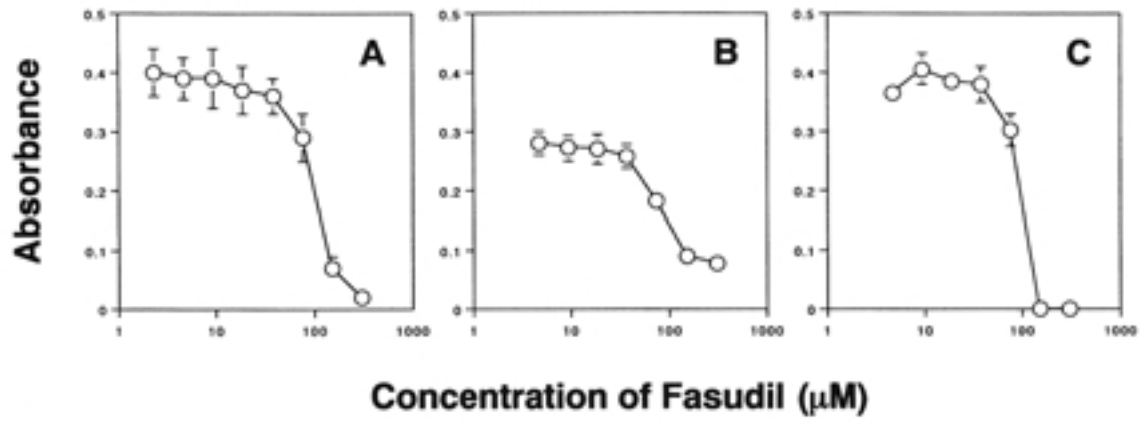

Fig.1. Cytotoxicity of fasudil for human lymphocytic cells. Jurkat cells (A) and unstimulated PBMCs (B) were cultured in CM in the presence of fasudil for 48 and 24 hours, respectively, and cell proliferation was evaluated by the WST-1 method. (C) PBMCs activated with PHA-P (1 μg/ml) were cultured in CM with 50 U/ml of rIL-2 and fasudil for 15 days (half volume of the medium was changed every 3 days), and the cell growth was measured as above. Triplicate samples were analyzed (absorbance at 450 nm), and the averages with error bars are shown.

dose of fasudil was basically similar to that in Jurkat cells. In activated PBMCs cultivated for 24 hours in complete medium (RPMI-1640 medium with 10% fetal calf serum, L-glutamine, 2-mercaptoethanol and antibiotics) (CM) (Fig.1 B) and for 15 days in CM supplemented with 50U/ml human recombinant interleukin-2 (rIL-2) (Serotec Ltd., Oxford, England) (Fig.1 C), the cytotoxic effect was seen at concentrations higher than 100 μM of fasudil.

Effect of fasudil on HIV-1 replication in T cell lines and PBMCs

On the basis of the cytotoxic data of fasudil described above, concentrations below 50 μM were used for the inhibition assay of virus replication.

First, the effect of fasudil on HIV-1 replication in T cell lines was monitored. As shown in Fig.2, in the two cell lines (CEMx174 and M8166), the virus growth kinetics in the presence (5 μM) or absence of fasudil were surprisingly similar. In a repeated experiment, essentially similar results were obtained (data not shown). Furthermore, cultivation of the two cell lines infected with various doses of virus in the presence of higher concentration of fasudil (50 μM) gave no significant effects with respect to virus production at 14 days post-infection (data not shown).

We then asked whether fasudil is effective against HIV-1 growth in PBMCs, the natural target cells. Fig.3 shows the replication kinetics of HIV-1 in PHA-P-stimulated PBMCs cultivated in rIL-2-supplemented CM in the presence of various concentrations of fasudil. On the peak day of RT production, the activity was reduced by 75%, 50%, 34%, and 33% of that of a control (no fasudil) at concentrations of 40, 20, 10, and 5 μM of fasudil, respectively. In a repeated experiment, essentially similar results were obtained (data not shown).

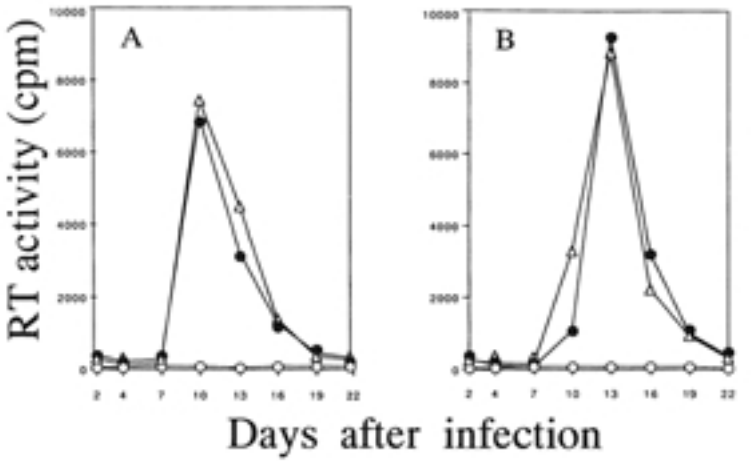

Fig.2. Effect of fasudil on HIV-1 replication in established cell lines. Cell-free viruses (3 x 10^5 RT units) obtained from HeLa cells transfected with pNL432 were inoculated into 2 x10^6 cells of CEMx174 (A) and M8166 (B), and RT production in the culture supernatants was monitored at indicated intervals. Symbols : ○, mock-infection ; ●, virus growth in the absence of fasudil ; △, virus growth in the presence of 5 μM of fasudil.

DISCUSSION

Our results here showed that fasudil has a suppressive effect on HIV-1 growth in activated PBMCs but not in established T cell lines. The basis for this observation is unclear. It has been shown that cell activation is required for HIV-1 replication in primary T lymphocytes (11, 12). Fasudil could inhibit the activity of protein kinase(s) required for HIV-1 replication in PBMCs but not in T cell lines. It is possible that fasudil affects the function of the *nef* gene which is known to be important for the viral replication in PBMCs (13, 14) and disease progression (15-17). A recent finding that fasudil inhibits Nef-dependent cytotoxicity (18) may be related to its suppressive effect of HIV-1 replication reported here.

Apart from the mechanism for the inhibitory effect, our results show that fasudil has a potential as a candidate for an anti-HIV-1 agent which can be administrated in

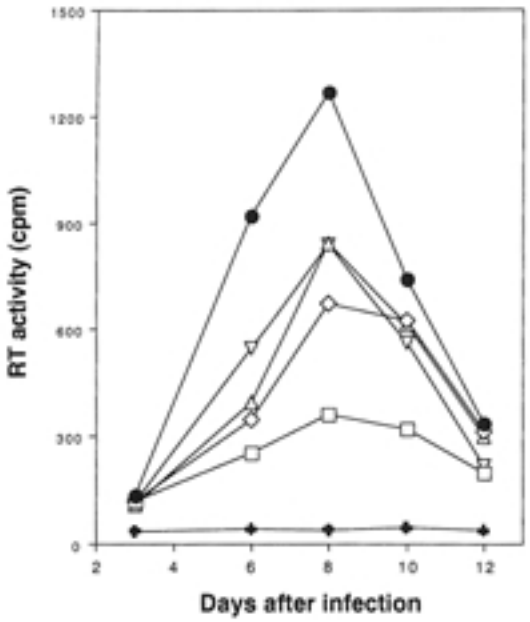

Fig.3. Effect of fasudil on HIV-1 replication in PBMCs. The activated PBMCs were infected with cell-free NL 432 prepared from transfected HeLa cells at a multiplicity of infection of 0.01. Virus titer was determined in MT-4 cells. The cultures were maintained in CM with 50 U/ml of rIL-2 and 40 μM(□), 20 μM(◇), 10 μM(△), 5 μM(▽), or absence (●) of fasudil. RT production in the culture supernatants was monitored at indicated intervals. +, mock-infection.

combination with inhibitors of the viral RT or protease. To further explore the possibility of fasudil as an anti-HIV-1 drug, animal experiments using simian immunodeficiency virus/macaque model system are to be done.

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