Inhibitory Effect of Newly Developed CXC-Chemokine Receptor 4 Antagonists on the Infection with Feline Immunodeficiency Virus

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(Received 16 November 2007/Accepted 29 September 2008)

ABSTRACT. CXC-chemokine receptor 4 (CXCR4) functions as a receptor for feline immunodeficiency virus (FIV). Although we previously found that a CXCR4 antagonist, T140, inhibited the FIV replication in vitro, it was not effective in cats infected with FIV because of its low stability in feline serum. To resolve this problem, several T140 derivatives have been developed. Here, we examined the inhibitory effect of TF14016 and TF14013, on the inhibition of FIV infection. These compounds were shown to significantly inhibit the syncytia formation in CXCR4-expressing cells after co-cultivation with FIV-infected cells and the replication of FIV in a feline lymphoid cultured cell line. These results indicated that TF14016 and TF14013 could be useful as antiviral drugs for cats infected with FIV.

KEY WORDS: CXCR4 antagonist, feline, FIV.

CXC-chemokine receptor 4 (CXCR4) is one of the co-receptors for human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) and necessary for the cell entry of both viruses [2, 3, 16, 23]. Therefore, blockade of CXCR4 has been expected to be a novel therapeutic strategy in HIV and FIV infections. Previous studies using a natural ligand for CXCR4, stromal cell derived factor-1 (SDF-1), revealed its inhibitory effect against HIV and FIV infections by blocking CXCR4 [4, 5, 8, 10, 16], but indicated its problematic side effect such as marked inflammation after injection in vivo [13]. Several CXCR4 antagonists, AMD3100, T22 and T140, were also shown to inhibit HIV infection [6, 7, 17] in cultured cells, but there is no report showing their clinical use because of their instability and strong side effect in vivo.

T22 is a derivative of a peptide derived from horseshoe crab blood cells [12]. This peptide consisting of 18-amino acid residues binds specifically to CXCR4 on human cells and inhibits the entry of HIV, however, it does not activate the target cells. Its derivative, T140, is unstable due to the cleavage of C-terminal Arg14 in serum and liver homogenate. However, the C-terminally amidated analogs of T140, TF14016 and TF14013, were shown to be bio-stable by virtue of stabilization of the Arg14 and possess high antiviral activity, low cytotoxicity, and in vivo stability [19]. In this study, we examined the inhibitory effect of TF14016 and TF14013 on the infection with FIV in cultured cells. These compounds were prepared as described previously [19] (Table 1). They were dissolved in water at 1 mM and kept in a freezer at –20°C until use for the assays.

First, we investigated whether TF14016 and TF14013 interact with feline CXCR4. The 3201 cells, which showed strong expression of cell surface CXCR4, were pretreated with 4 µM of these compounds on ice for 30 min. Then, these cells were reacted with an anti-CXCR4 monoclonal antibody (clone 44717) (R & D systems, Minneapolis, MN) in flow cytometric analysis (FCM) buffer (2% fetal calf serum in PBS) on ice for 30 min. The cells were washed twice with FCM buffer and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody for 30 min on ice. The cells were washed twice with FCM buffer and analyzed with a flow cytometer (FACSCalibur) (Becton Dickinson Immunocytometry systems, San Jose, CA). The fluorescence intensity of 3201 cells detected by clone 44717 was remarkably diminished when the cells were preincubated with both of the compounds (Fig. 1). Clone 44717 was shown to bind to the extracellular loop 2 (E-L2) region of human and feline CXCR4 in the N-terminal domain [1]. The previous report showed that T140 compounds physically bind to E-L2 region of human CXCR4 [22]. Moreover, the E-L2 region was shown to be the most important site in feline CXCR4 for the interaction with FIV envelope protein [23]. Consequently, it is conceivable that the masking of E-L2 region with the T140 analogs results in

Table 1. Amino acid sequence of T140 analogs

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<th>Amino acid sequence of T140 analogs</th>
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<tr>
<td>TF14016 4F-benzoyl-Arg-Arg-Nal-Cys-Try-Lys-4DLys-Pro-Tyr-Arg-Cit-Cys-Arg-NH₂</td>
</tr>
<tr>
<td>TF14013 4F-benzoyl-Arg-Arg-Nal-Cys-Try-Lys-4DGlupro-Tyr-Arg-Cit-Cys-Arg-NH₂</td>
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NOTE: Virology
HeLa cells were seeded in Dulbecco’s Modified Eagle’s Medium (DMEM) and cultured overnight to form monolayers. On the following day, TF14016 or TF14013 (4 µM each) was added, and the cells were cultured at 37°C for 1 hr. Then, CRFK/FIV cells [15] were added into each of the wells. After 24 hr, the cells were fixed with methanol and stained with Wright and Giemsa solutions. After co-cultivation with CRFK/FIV cells, a number of syncytia were observed as reported by Willet et al. [23]. When the HeLa cells were pretreated with either TF14016 or TF14013, the syncytia formation in HeLa cells after co-cultivation with CRFK/FIV cells was remarkably inhibited (Fig. 2). Syncytia formation inhibition rates by the treatment with TF14016 and TF14013 (4 µM) were 98.1% and 96.2%, respectively, which were calculated as [(number of syncytia in the presence of the compound–number of spontaneously formed syncytia)/(number of syncytia in the absence of the compounds–number of spontaneously formed syncytia) × 100].

Inhibitory effect of the CXCR4 antagonists on the replication of FIV was examined in Kumi-1 cells which were highly permissive to FIV infection [9]. Prior to the experiment, the CXCR4 antagonists were shown to induce no cytotoxic effect on Kumi-1 cells at their concentrations up to 4 µM. Kumi-1 cells (2 × 10^6 cells/ml) were first treated with each of the CXCR4 antagonist compounds, TF14016 and TF14013 (4 µM), at 37°C for 1 hr, and then inoculated with a subtype A FIV strain, Sendai-1 [11] (final concentration of reverse transcriptase (RT) activity, 50,000 cpm/ml).
After adsorption at 37°C for 3 hr, the cells were washed twice with PBS and resuspended to a final cell density of $5 \times 10^5$ cells/ml. The cells were cultured in RPMI-1640 medium supplemented with 100 U of human recombinant interleukin-2 (Pharma Biotechnologie, Hannover, Germany) per ml. They were passaged 3 days later to dilute the cell density to $5 \times 10^5$ cells/ml and were added with each of the fresh compounds. The cell-free supernatants were harvested 6 days after inoculation with FIV to measure RT activity as described previously [8]. When the Kumi-1 cells were pretreated with TF14016 (4 $\mu$M) or TF14013 (4 $\mu$M), the replication of FIV in Kumi-1 cells was significantly inhibited as shown by the decrease of RT activity in their culture supernatants (Fig. 3a). The TF14016 was shown to decrease the RT-activity in the supernatants of the FIV-infected Kumi-1 cells in a dose-dependent manner (Fig. 3b).

The C-terminally amidated analogs of T140 as used in this study were shown to have a potential efficacy against HIV infection [19] as well as rheumatoid arthritis and tumor metastasis mouse models [18, 20]. Additionally, previous studies demonstrated that these compounds were considerably stable in feline serum [21] and effectively inhibited feline SDF-1-induced migration of a feline mammary tumor cell line [14]. In this study, these compounds were shown to significantly inhibit FIV replication at the cell entry level. Thus, it is reasonable to suppose that the administration of these new T140 analogs may exert their antiviral effect in cats infected with FIV. However, because the inhibitory effect of T140 analogs against FIV infection was observed at concentrations more than 400 nM, clinically achievable blood levels remain as a matter to be investigated. Further study is needed to examine the in vivo bioavailability, efficacy, and toxicity of the new T140 analogs before their clinical application in cats infected with FIV.

ACKNOWLEDGEMENT. This study was supported by grants from the Japan Health Science Foundation and the Ministries of Education, Science, Sports and Culture, and Health and Welfare of Japan.

REFERENCES


