Development and evaluation of a novel peptide based therapy for feline leukemia virus (FeLV)-infection in domestic cats

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Aim

Our aim was to find potential antiviral peptides to inhibit infection of feline cell lines by FeLV.

Introduction

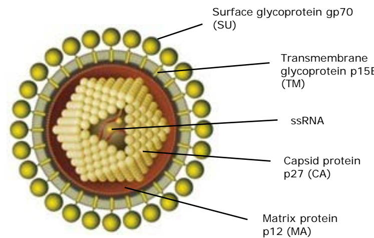
FeLV

The feline leukemia virus is a worldwide occurring gammaretrovirus of pet cats and some related small felids (1). Infection with FeLV may cause persistent viremia and associated fatal diseases in a portion of infected cats (2). Despite the commercial availability of several effective vaccines, FeLV is still prevalent in cat populations. So far, there is no therapy available for clinical use.

Inhibition of viral entry

The entry step of retroviruses involves fusion of the viral and host cell membranes (3). Peptides which effectively inhibit membrane fusion and thus viral entry have been identified for human and animal lentiviruses (4, 5, 6, 7). They mimic parts of the viral transmembrane (TM) glycoprotein.

Figure 1 A



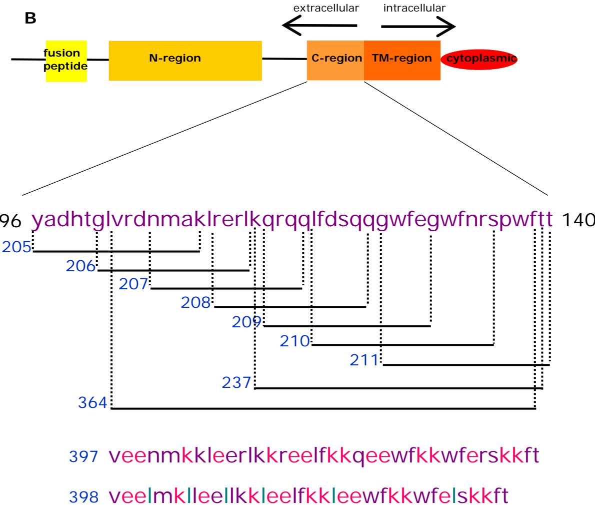


Fig. 1

A. Schematic representation of the FeLV virion particle.

B. The FeLV p15E (TM) glycoprotein.

Potential antiviral peptides mimic parts of the viral TM. Peptides 205-211 are 15, 237 is 25 and 364 is 37 amino acids long. 397 and 398 (both 37 amino acids) are the modified versions of 364, with Glutamate (e) and Lysine (k) replacing certain amino acid residues to increase solubility and stability. Leucine (l) may help to create strong bonds to target amino acids.

Discussion

In contrast to short peptides, the long peptides 364, 397 and 398 effectively inhibit viral replication *in vitro*. This might be due to higher stability, alphahelicity and solubility of long peptides.

Inhibition of replication is obtained when virus is added prior to the peptide (virus inhibition assay b). If the virus is mixed with the test peptide and added to the cells (assay a), there is no inhibitory effect.
We conclude that this observation might be due to an unknown inhibitory mechanism of our peptides which is directed rather to cell escaping virus than to cell invading virus.

A therapy might become important for humans too, especially in xenotransplantations during which unknown gammaretroviruses may lead to severe infections.

Results

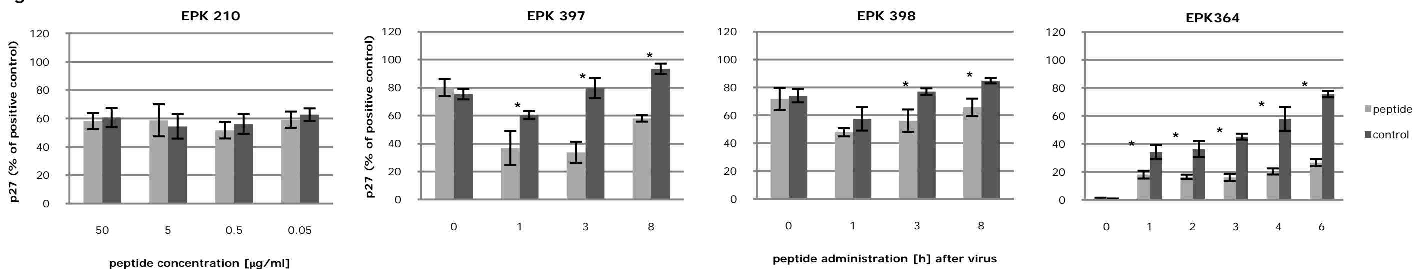


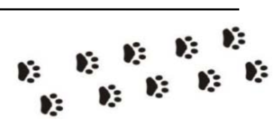
Fig. 2: The inhibitory effect of the synthetic peptides on the replication of FeLV. **A:** Done with virus inhibition assay a. **B, C** and **D**: Done with virus inhibition assay b. Virus replication is given in % of the positive control. Peptide concentration for B, C and D: 50 µg/ml. Asterisks indicate significant difference from the value in the absence of a peptide. Data are shown as means (n=3).

Summary

Long synthetic peptides derived from the FeLV TM significantly inhibited viral replication *in vitro*.

In future, potent peptides might be tested *in vivo*, too.

Materials & Methods



Cells
A feline embryonic fibroblast cell line (FEA) susceptible to FeLV was used.
Peptides
Eleven peptides derived from the FeLV-A TM (gene bank no. AAA93093) have been designed (Fig. 1B) and tested.
FeLV-A
The molecularly cloned FeLV subtype A/Glasgow-1 (8) was used in all assays.

Virus inhibition assays
a: Cell-free virus (10 x $TCID_{50}$) was mixed with the test peptides to final concentrations of 50, 5, 0.5 and 0.05 µg/ml, or with diluents as a control. The mixtures were immediately added to 5x10³ cells/well of a 96-well microtiter plate. After 2-5 hrs at 37°C, inocula were rinsed and replaced with 200 µl fresh culture medium.
b: Virus (10 x $TCID_{50}$) was added to 5 x 10³ cells/well of a microtiter plate. After 0-8 hrs incubation at 37°C, inocula were rinsed and the peptide (or diluent as a control) was added to final concentrations of 50, 5, 0.5 and 0.05 µg/ml.

Both assays were stopped after 7 additional days. The supernatants were tested by enzyme-linked immunosorbent assay (ELISA) (9) for the presence of p27 virus antigen.

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