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Short communication

Comparative evaluation of the activity of antivirals towards feline immunodeficiency virus in different cell culture systems

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Abstract

Influences of the cell system on observed EC_{50} values of different agents against feline immunodeficiency virus (FIV) were assessed. The activity of various nucleoside reverse transcriptase inhibitors (NRTI) against a lymphotropic FIV strain was evaluated using monocultured thymocytes and a DC–thymocyte coculture. In the second set of experiments activity of carbohydrate binding agents (CBA) towards FIV strains derived from different cell lines (e.g. Crandall feline kidney cells (CRFK) and thymocytes) was compared. We examined three different FIV-based antiviral evaluation systems and obtained marked differences in EC_{50} values, especially for CBA entry inhibitors. Our study confirms and extends earlier observed differences between cell systems used for the evaluation of the activity of antivirals towards FIV.

Keywords: Antiviral testing; NRTI; Plant lectins; FIV; In vitro assay

Feline immunodeficiency virus (FIV) mimics human immunodeficiency virus (HIV) infections with respect to pathogenesis, genome organization and provoked immune responses (Bendinelli et al., 1995; Willett et al., 1997a). On this basis FIV infections have been used to test antiviral compounds (in vitro and in vivo) for their potential activity towards human immunodeficiency virus (HIV). Thus, the antiviral activity of nucleoside reverse transcriptase inhibitors (NRTI) (Egberink et al., 1990; Hartmann et al., 1992; North et al., 1989; Vahlenkamp et al., 1995), entry inhibitors (Balzarini et al., 2004; Egberink et al., 1999; Tanabe-Tochikura et al., 1992) and protease inhibitors (Lee et al., 1998; Wlodawer et al., 1995) were previously studied for their inhibiting potency against FIV.

In vitro the evaluation of anti-FIV activity is generally performed on lymphocytic cells, infected with a lymphotropic FIV strain. Alternatively, a fibroblast cell line (Crandall feline kidney cells, CRFK) either freshly or persistently infected with CRFK tropic FIV strains is used. When the EC_{50} values of AZT (zidovudine) towards FIV were determined in these cell systems, differences of up to 80-fold were observed (Vahlenkamp et al., 1995). These observations point to a direct effect of the particular cell system used on drug efficacy results, which could in some cases lead to the unjustified rejection of compounds as candidate antivirals. Ideally, the in vitro model system resembles the in vivo situation closely, thereby enhancing the predictability of antiviral activity in patients (Pauwels, 2006). To further assess the influences of the cell system on the observed EC_{50} values of different agents against FIV the present study was performed.

In a first set of experiments, two specific entry inhibitors, i.e. carbohydrate binding agents *Galanthus nivalis* agglutinin (GNA) and *Hippeastrum* hybrid agglutinin (HHA) (Van Damme et al., 1998), previously shown to block HIV infection (Balzarini, 2006), were evaluated for their ability to inhibit FIV entry using established lymphocytic-, PBMC- and CRFK-based culture systems. FIV uses different entry mechanisms to infect these host cells. To enter thymocytes and PBMC, the virus uses CD134 (de Parseval et al., 2004) as its primary receptor, whereas only the chemokine coreceptor CXCR4 (Willett et al., 1997b) is needed to efficiently infect CRFK. In a second set of experiments we evaluated NRTIs such as PMEA (adefovir) (Egberink et al., 1990), the *R* enantiomer of (*R*)-PMPDAP (Vahlenkamp et al., 1995) and AZT (zidovudine) (Vahlenkamp et al., 1995), in a new dendritical cell (DC)–thymocyte coculture system. The presence of DCs cultured from bone marrow of SPF cats

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in the DC–thymocyte cocultures has recently been shown to enhance FIV infections (van der Meer et al., 2007). Since DCs are involved in the early pathogenesis of retrovirus infections (Obert and Hoover, 2002; van Kooyk and Geijtenbeek, 2003), testing the activity of antivirals in this cell model is obviously of special importance, particularly for those NRTI known already to inhibit FIV in thymocyte monocultures.

Plant lectins like GNA and HHA target the mannose residues of N-linked oligosaccharides attached to the viral envelope glycoproteins (Balzarini, 2006). It is, however, well-known that host cells are important in determining the structure of the glycan structures present on the viral glycoproteins (Rademacher et al., 1988). FIV Utrecht 113 strains FIV-113$_{Th}$, FIV-113$_{PBMC}$ and FIV-113$_{CRFK}$ were propagated in their respective host cells, i.e. thymocytes, peripheral blood mononuclear cells (PBMC) and CRFK. One hundred $TCID_{50}$ (50% tissue culture infective dose) of each FIV was used to infect their homologous host cells in the presence of various concentrations of GNA or HHA. To determine and quantify infection of the cells, viral antigen released into the culture supernatant was evaluated at 6 days post-infection by p24 ELISA (Egberink et al., 1992). Neither GNA nor HHA showed cytotoxicity towards the cell types evaluated which was determined in parallel performed experiments. Our results (Table 1) indicated that FIV-113$_{CRFK}$ was very sensitive to the inhibitory effect of plant lectins in CRFK cultures (EC_{50}: 0.8×10^{-3} to 1.4×10^{-3} μM). This high inhibitory potency was in sharp contrast to the low sensitivity of FIV-113$_{Th}$ in thymocyte cultures (EC_{50}: 0.3–1.8 μM). When the antiviral activities of these lectins were subsequently determined in PBMC derived FIV-113$_{PBMC}$ the lectin efficacy was comparable to the values obtained in the FIV-113$_{Th}$–thymocytes infection system.

These results suggested that the oligosaccharides carried by FIV-113$_{Th}$ and FIV-113$_{PBMC}$ differ from those present on FIV-113$_{CRFK}$. FIV-113$_{CRFK}$ and FIV-113$_{Th}$ envelope proteins differ only in a single amino acid, not related to glycosylation (Vahlenkamp et al., 1997). We therefore hypothesized that the host cell glycosylation machinery influenced the maturation of the glycan structures, thereby determining the differences in FIV lectin sensitivity. To confirm this we attempted several times to propagate FIV-113$_{CRFK}$ on thymocytes in order to obtain FIV-113$_{CRFK}$ with a thymocyte-like glycosylation pattern on its envelope glycoproteins. All these attempts failed. Even though the p24 capsid protein production as assessed by ELISA was high, infectious progeny virus was undetectable by virus titration on thymocytes. Interestingly, this observation indicates that FIV-113$_{CRFK}$ is able to infect thymocytes and even initiate replication in these cells, without the production of infectious thymotropic virus. The high sensitivity of CRFK-tropic viruses to plant lectins might be related to the presence of specific glycan structures (i.e. high-mannose type glycans) on their envelope when propagated in CRFK cultures. The reason for the much lower activity of the CBA against FIV in PBMC and thymocytes is still unclear. It would be interesting to determine the nature of the N-glycans of the envelope glycoproteins of the different virus strains to establish whether our hypothesis is correct. Alternatively, the observed differences might be related to specific properties of their earlier-mentioned entry mechanism. When the fusion time of FIV-113$_{CRFK}$ on CRFK would be significantly longer compared to that of FIV-113$_{Th}$ or FIV-113$_{PBMC}$, the lectin binding sites on the viral envelope glycoproteins might be exposed much longer to the carbohydrate recognition domains of HHA and GNA. Hence, inhibition of FIV infection by HHA or GNA in CRFK would be more efficacious. Based on the FIV-113$_{CRFK}$ results plant lectins are promising antivirals against FIV. However, based on the FIV-113$_{PBMC}$ and FIV-113$_{Th}$ data, the high EC_{50} values are rather disappointing in terms of antiviral efficacy of the plant lectins.

Table 1
The antiviral activity of GNA and HHA towards FIV Utrecht 113 derived from different cell types

Lectin	FIV-113$_{CRFK}$	FIV-113$_{Th}$	FIV-113$_{PBMC}$
GNA (EC_{50})	$1.4 \times 10^{-3} \pm 1.6 \times 10^{-3}$	1.8 ± 0.06	1.4 ± 0.08
HHA (EC_{50})	$0.8 \times 10^{-3} \pm 0.8 \times 10^{-3}$	0.3 ± 0.06	0.4 ± 0.02

FIV-113$_{CRFK}$ was derived from CRFK ATCC, FIV-113$_{Th}$ was derived from thymocytes and FIV-113$_{PBMC}$ from peripheral blood mononuclear cells harvested from an SPF cat. The EC_{50} is expressed in μM (±S.D.).

The antiviral activity of NRTI (PMEA, (*R*)-PMPDAP and AZT) in a thymocyte monoculture was compared to that in the DC–thymocyte coculture using the thymotropic FIV strain. In these experiments a 1:10 DC–thymocyte ratio was used. FIV-113$_{Th}$ (100 $TCID_{50}$) was used to infect the thymocyte cell cultures in the presence of various concentrations of the NRTI. The infection was evaluated at 6 days post-infection by determining p24 antigen present in the supernatant using the FIV p24-specific ELISA. Statistical analyses were performed using a Student's *t*-test. The cytotoxicity was evaluated in parallel in which none of the NRTIs induced cytotoxicity in the concentration range shown in Fig. 1. In both in vitro systems PMEA, (*R*)-PMPDAP and AZT were significantly active against FIV (Fig. 1; for (*R*)-PMPDAP, results not shown). PMEA and (*R*)-PMPDAP showed similar antiviral activity curves in thymocytes and DC–thymocyte cultures. The EC_{50} values of PMEA and (*R*)-PMPDAP determined in these cell cultures were not significantly different (EC_{50} PMEA: DC–thymocyte 0.65 ± 0.20 μg/ml; thymocytes 0.42 ± 0.17 μg/ml; EC_{50} (*R*)-PMPDAP: DC–thymocyte 0.07 ± 0.02 μg/ml; thymocytes 0.11 ± 0.04 μg/ml). However, AZT showed a 6-fold ($p < 0.01$) less inhibitory potency in DC–thymocyte cocultures than in thymocyte monocultures (EC_{50} AZT: DC–thymocyte 0.98 ± 0.62 μg/ml; thymocytes 0.15 ± 0.07 μg/ml). For PMEA and AZT the EC_{50} values are the average ± S.D. of six independent tests, for (*R*)-PMPDAP they are the result of three independent tests. Previously, we showed that addition of DC to thymocytes induced proliferation of the thymocytes (van der Meer et al., 2007). This will probably give rise to differences of drug metabolism (Gao et al., 1993, 1994) due to an enhanced phosphorylating nucleoside kinase activity in the stimulated thymocytes. As AZT activity is dependent on cellular phophorylation its antiviral activity would be expected to increase as well. However, other phenomena may partly counteract the AZT efficacy. Indeed, FIV also replicates to markedly

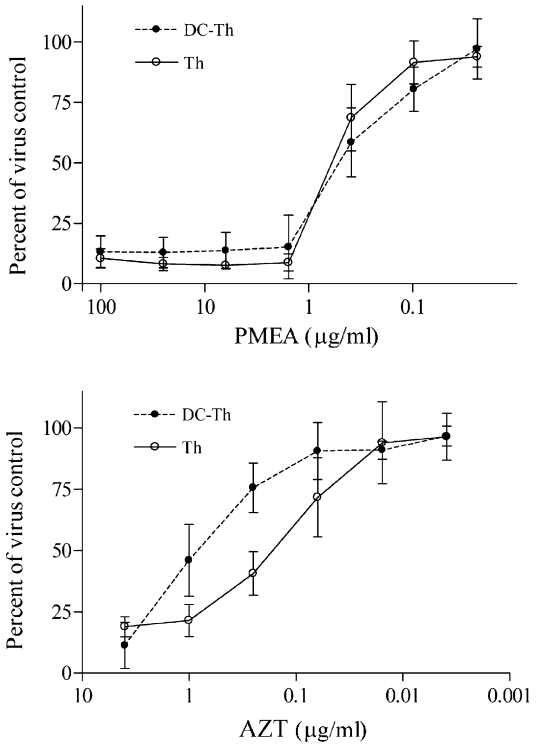

Fig. 1. The influence of PMEA or AZT on feline thymocyte monocultures or feline dendritical cell–thymocyte cocultures. The *X*-axis represents the concentration antiviral compound, the *Y*-axis indicates the normalized p24 antigen production as determined by ELISA (Egberink et al., 1992). A dot represents the average of six independent tests. The whiskers indicate the standard deviation.

higher levels in activated thymocytes, and the expansion of the endogenous dideoxynucleoside 5′-triphosphates pools, in particular dTTP, will compete with the AZT-5′-triphosphate levels (Van Herrewege et al., 2002).

The changed balance between these phenomena in DC-stimulated thymocytes may explain the eventual decrease in antiviral efficacy of AZT, but not PMEA and (*R*)-PMPDAP. The metabolism of the latter drugs is indeed known to be much more independent on the metabolic condition of the cells than AZT. Acyclic nucleoside phosphonates like PMEA and (*R*)-PMPDAP have a long-lasting antiretroviral activity due to the relatively slow metabolism of the phosphorylated PMEA derivatives and, especially, to the relatively long intracellular half-life of the active metabolites (the diphosphorylated analogues). The intracellular breakdown of AZT-TP is much faster (Balzarini, 1994; Veal and Back, 1995), which might partly explain the efficacy differences observed.

In conclusion, we examined three different FIV-based antiviral evaluation systems and obtained marked differences in EC_{50} values, especially for CBA entry inhibitors. Cell cultures used for antiviral testing are in most cases based on similar culture systems as routinely used to propagate the viruses. Our study confirms and extends earlier observed differences between cell systems used for the evaluation of the activity of antivirals towards FIV (Vahlenkamp et al., 1995). For the correct interpretation and extrapolation of the obtained EC_{50} values to the in vivo situation the degree of similarity between the used in vitro models and the actual in vivo situation must be taken into consideration.

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