Short communication

Inhibition of Feline leukemia virus replication by the integrase inhibitor Raltegravir

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A R T I C L E   I N F O

Article history:
Received 19 January 2011
Received in revised form 21 March 2011
Accepted 31 March 2011

Keywords:
Integrase inhibitor
Feline leukemia virus
Antiretroviral therapy
Progressive infection
Raltegravir
Retrovirus

A B S T R A C T

The oncogenic gammaretrovirus Feline leukemia virus (FeLV) has been the leading cause of death among domestic cats until the introduction of efficient diagnostics and vaccines in the late 1980s. So far, no efficient treatment for viremic animals is available. Hence, use of the FeLV model to evaluate antiretroviral therapies applied to HIV is a timely task. The efficacy of the integrase inhibitor Raltegravir, which is widely used for the treatment of HIV in humans, has been assessed in vitro for the FeLV-A/Glasgow-1 strain. EC50 values for FeLV-A inhibition in feline cell lines are in the range of that observed for HIV and xenotropic murine leukemia virus-related gammaretrovirus. Therefore, Raltegravir may be a potential therapeutical agent for felids with progressive FeLV infection.

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1. Introduction

Thirty to forty percent of cats infected with the gammaretrovirus Feline leukemia virus (FeLV) have a course of the disease known as progressive FeLV infection (Hofmann-Lehmann et al., 2008) and shed the virus continuously under experimental conditions (Gomes-Keller et al., 2009). The prognosis for progressively infected cats is poor, and 70–90% of those cats die within 18 months to 3 years after developing disease symptoms that range from immune suppression, to anemia and lymphoma/leukemia (Lutz et al., 2009). After the introduction of reliable, sensitive diagnostics and efficient vaccines in the late 1980s, the prevalence of the infection dropped significantly, but is still relatively high in some countries, especially among sick cats, and in multi-cat households with no specific preventive measures in place, where it may exceed 20% (Akhtardanesh et al., 2010; Blanco et al., 2009; Duarte et al., 2010; Gleich et al., 2009; Lutz et al., 2009). Furthermore, FeLV is a threat to the survival of the most endangered felid, the Iberian lynx (Meli et al., 2009). Thus, the assessment of the effect of highly efficient antiretroviral therapies (ARTs) on FeLV in vitro as well as in vivo would be more than welcome to alleviate the consequences of the infection in domestic cats and other felids. The efficacy of antiretroviral drugs is low in FeLV infection, and many have severe side effects in cats (Hartmann et al., 1992). There are only a few controlled studies that have demonstrated some effect. Feline interferon omega inhibits FeLV replication in vitro, and treatment of viremic cats with this cytokine has been shown to significantly improve clinical scores and extend survival times (de Mari et al., 2004). However, no viral parameters were measured throughout this study to support the hypothesis that interferon-omega actually exerted an antiviral effect. The nucleoside analogue 3’-azido-2’,3’-dideoxythymidine (AZT) effectively inhibits FeLV replication in vitro, and in vivo in experimental infections; but its toxicity may cause unwanted side effects (e.g., non-regenerative anemia) and is therefore not recommended as a first line of therapy for progressively infected domestic cats (Lutz et al., 2009). Other ARTs used for the treatment of e.g. lentiviral infections in humans are
not equally efficient for gammaretroviruses. Indeed, only four of the compounds listed as standard therapy recommendation for HIV patients that have not experienced any antiretroviral therapy (Office of AIDS Research Advisory Council (OARAC) et al., 2009) seem to be efficient in vitro against the xenotropic murine leukemia virus-related virus XMRV (Paprotka et al., 2010; Singh et al., 2010; Smith et al., 2010). Of 32 compounds tested by Singh and co-workers only AZT, Tenofovir, Efavirenz (reverse transcriptase inhibitors) and Raltegravir (integrase inhibitor) were effective against XMRV, with Raltegravir being the best inhibitor of replication (Singh et al., 2010). In addition, Efavirenz was only effective near the critical cytotoxic concentration and Tenofovir was more cytotoxic than AZT when used in concentration yielding comparable effects. HIV protease inhibitors were not active against XMRV. Thus, of the compounds currently recommended for human HIV therapy, Raltegravir (or, alternatively, other integrase inhibitors that are not yet on the market, like Elvitegravir (Shimura et al., 2008), MK-2048 (Bar-Magen et al., 2010) or S/GSK1349572 (Charpentier et al., 2010; Min et al., 2010; Prada and Markowitz, 2010; Song et al., 2010)) may be considered for the treatment of FeLV-infected felids in addition to AZT.

2. Materials and methods

To determine the inhibitory concentration of Raltegravir in vitro, three feline cells lines (QN10 (a feline fibroblast cell line carrying a defective murine sarcoma virus with the mos oncogene), feline embryonic fibroblasts (FEA) and Crandell Reese feline kidney cells (CrFK)) were cultured in 24-well plates (TPP, Trasadingen, Switzerland), 10,000 cells/well, in RPMI medium containing 10% FCS (both Sigma–Aldrich, Buchs, Switzerland), and 1× Penicillin–Streptomycin (Sigma–Aldrich) for 24 h with polybrene (hexadimethrine bromide, Sigma–Aldrich, 4 μg/ml concentration) and seven different concentrations of Raltegravir (SelleckChem, Houston, TX, USA). Concentration range was of 0.196 nM to 3 μM. Fig. 1. Raltegravir was diluted in RPMI medium from a stock solution of 50 mM in DMSO) in triplicate wells, and infected with 5000 focus forming units FeLV-A/Glasgow-1 (corresponding to 10× 50% tissue culture infectious doses, TCID50) for 2 h before washing the cells 1× with PBS. Cells were consequently incubated for 7 days (QN10: 5 days) in 500 μl RPMI medium with daily media change and the same Raltegravir concentrations as above. Potential cytotoxicity of Raltegravir was monitored using the CellTiter-Blue Cell Viability and Apo-ONE Homogeneous Caspase-3/7 Assay (Promega, Madison, WI, USA) according to manufacturer’s instructions. Two hundred microliters of cell culture supernatant were collected, centrifuged in a table-top centrifuge at maximal speed for 2 min to pellet cells and debris, and viral RNA extracted using the MagnaPure LC TNA extraction kit (Roche, Basel, Switzerland) according to manufacturer’s instructions. FeLV RNA was quantitated by real-time RT-PCR as described (Tandon et al., 2005) and inhibition kinetics in QN10 cells determined by sigmoid dose–response curve fitting using GraphPad Prism version 5.0 (GraphPad Inc., La Jolla, CA, USA).

3. Results

Concentration of up to 3 μM Raltegravir had no appreciable effect on cell viability nor induced apoptosis. Raltegravir effective 50% inhibitory concentrations (EC50) were of 7.6 nM in QN10, of 1.3 nM in FEA and of 2.2 nM in CrFK cells (Fig. 1).

4. Discussion

The high degree of conservation along lentiviruses, betaretroviruses, gammaretroviruses, and alpharetroviruses of integrase active sites, of the integrase DNA binding site and of the potential amino acid residues known to confer resistance to integrase strand transfer inhibitors (Koh et al., 2011), suggests that FeLV may be highly sensitive to the integrase inhibitor Raltegravir. Importantly, the estimated EC50 values are in the range or below of what observed e.g. for HIV and XMRV (Le et al., 2010; Serrao et al., 2009; Singh et al., 2010; Smith et al., 2010), and well below the minimal plasma concentrations that can be found e.g. in humans (Ter Heine et al., 2010). In QN10 cells, inhibition seems to reach a plateau at concentrations higher than 100 nM, when the inhibition of replication has reached >99.9% (Fig. 1). However, incubation with 35 μM Raltegravir (not shown) completely abolished replication, suggesting that the plateau may be the result of experimental variations. The efficiency of Raltegravir at very low doses renders it interesting for an application in vivo, since Raltegravir is partly eliminated as glucuronide (Kassahun et al., 2007), a pathway that is not very efficient in cats, increasing the risk of toxicity due to drug accumulation. In vivo testing followed by treatment interruption will be the true benchmark for the use of Raltegravir in veterinary medicine. Indeed, it has long been known that some cats, after a transient period of viremia of up to 9 months, can efficiently suppress viral replication and fully recover from viremia (Lutz et al., 1980), indicating the possibility that if viral loads can be
significantly reduced for long enough, the immune system of the cat may be able to overcome the infection. We speculate that prolonged Raltegravir treatment may induce the same state and the antiretroviral drug may be subsequently withdrawn without viral rebound. This was observed e.g. in some HIV patients to whom potent ART had been given early during primary HIV infection and for an extended period. In these patients, control of viral replication is associated with a low viral reservoir that may not be replenished after ART interruption (Hocqueloux et al., 2010). A similar observation in FeLV infected cats treated with Raltegravir would be of primary importance for several reasons. First, long-term compliance to treatment is an issue for domestic cats, which have to rely on their owner for the administration of the medications. Alas, the financial aspect has to be considered as well: the price of an antiretroviral therapy may become prohibitive if the drug has to be administered continuously. And last, the application of antiretroviral therapies to wild felids heavily affected by FeLV, such as the Iberian lynx (Meli et al., 2009), will be really useful only if, after treatment, the animal has fully recovered from infection and can be released into the wild.

5. Conclusion

In conclusion, we demonstrated that Raltegravir-mediated FeLV inhibition kinetic is potentially compatible with a use in vivo for the treatment of progressively infected felids.

Acknowledgments

Laboratory work was performed using the logistics of the Center for Clinical Studies at the Vetsuisse Faculty of the University of Zurich. The project was supported by the University of Zurich, Switzerland and by financing the operational activities of the Clinical laboratory of the Vetsuisse Faculty, Zurich. We also acknowledge support from the Medica Foundation, Chur, Switzerland. We thank Dr. O. Jarrett, who kindly provided the FeLV-A/Glasgow-1 virus stock. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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