Antiretroviral Agents Inhibit Infection of Human Cells by Porcine Endogenous Retroviruses

S. K. POWELL, 1 M. E. GATES, 1 G. LANGFORD, 2 M.-L. GU, 1 C. LOCKEY, 1 Z. LONG, 1 AND E. OTTO 1*

Genetic Therapy, Inc., a Novartis Company, Gaithersburg, Maryland 20878, 1 and Imutan, Ltd., a Novartis Pharma AG Company, Cambridge, CB2 2YP United Kingdom 2

Received 18 April 2000/Returned for modification 22 June 2000/Accepted 25 August 2000

The efficacy of antiretroviral drugs against porcine endogenous retroviruses (PERV) that may be harbored in pig organs intended for transplantation was examined in human cells in vitro. The nucleoside analogs zidovudine and dideoxynosine were found to effectively inhibit PERV replication.

The promise of unlimited supplies of organs for transplantation makes advances in xenotransplantation an exciting area of medicine. However, concerns have been raised about the possible transmission of disease from donor animals to human patients. In particular, pigs which may be suitable for use as donors of hearts and other organs have been found to contain endogenous retroviruses (porcine endogenous retroviruses [PERV]). While the analysis of humans treated with living pig tissues as well as that of monkeys transplanted with pig organs found no evidence of any cross-species transmission of PERV in vivo (10, 13, 15, 16; Z. Long, personal communication), the ability of PERV to infect human cells in vitro has been demonstrated by several groups (13, 15, 16), suggesting that there may be a risk of patient infection during xenotransplantation. To identify treatments that could minimize the risk from such infections, the efficacy of antiretroviral drugs against PERV was determined in human cells in vitro.

To minimize variability between experiments, a single high-titer stock of PERV generated by infection of human 293 cells with culture supernatant from porcine PK-15 cells was used for all drug efficacy studies. For each treatment, 5 × 10^5 293 cells were plated in six-well plates and infected with 1 ml of viral supernatant in a total volume of 2 ml of medium containing a final concentration of 8 μg of polybrene per ml; the multiplicity of infection under these conditions was approximately 1. Cells were maintained for 3 weeks in the presence or absence of antiretroviral drugs. To determine if the antiretroviral drugs had toxic effects on 293 cells that might interfere with virus infection, uninfected cultures were carried for three passages in the presence of the drugs and counted at each passage. No difference was observed between control and drug-treated cells (data not shown).

The level of PERV virions in supernatant collected from these cultures was quantified using a PCR assay that specifically detects PERV RNA. Real-time PCR was performed and detected using the Taqman chemistry and the 7700 sequence detection system (PE Biosystems) as previously described (11). The primer sequences used for the RNA-specific PCR are as follows: 5′-GAAACATCGATGACAAGCTTAGGTATCGAT AACAGCGTTGTTGGTGTGGTCA-3′ (reverse PCR primer) at a final concentration of 300 nM; 5′-AGCTCCGGGA GGCCCTACTC-3′ (forward PCR primer) at a final concentration of 300 nM; and 5′-GAACATCGATGACAAGCTTAGGTATCGAT AACAGCGTTGTTGGTGTGGTCA-3′ (reverse transcriptase primer) at a final concentration of 300 nM. The probe used to detect PERV-specific products was 5′-(6-carboxy-fluorescein)-CCACCGTGCAAGGAACCTCGAG ACT-(6-carboxy-tetramethyl rhodamine)-3′ at a final concentration of 100 nM. To quantitate viral load, amounts following interpolation from the standard curve. Levels of PERV infection achieved in control 293 cells which were not treated with antiretroviral agents ranged from 10^3 to 10^7 copies per cell.

The results of dose-response experiments with the nucleoside analogs zidovudine (AZT), dideoxynosine (ddI), lamivudine (3TC), and stavudine (d4T) and the protease inhibitor indinavir are shown in Fig. 1. AZT is a potent inhibitor of PERV replication in 293 cells, with a 50% inhibitory concentration (IC50) of approximately 0.25 μM. ddI also inhibited PERV, with an IC50 of approximately 1 μM. These concentrations are similar to those reported in vitro studies of human immunodeficiency virus type 1 (HIV-1) (4, 5, 7), and some degree of cross-resistance between these two viruses has been noted in clinical settings. PERV and MLV are identical in this region but have several residues that differ from HIV-1, most notably at position 75, where the amino acid change from valine to threonine has been associated with resistance. In PERV and MLV, resistance to 3TC and d4T; sequence comparisons revealed that the mutations associated with specific drug resistance patterns in HIV-1 occur naturally in the reverse transcriptase (RT) of MLV (14). Comparisons of PERV, MLV, and HIV RT using published sequences show that the RT of PERV is highly related to that of MLV, and sequence comparison in the region associated with resistance to 3TC reveals that like MLV, PERV also contains the single base pair change associated with resistance to this nucleoside analog (Fig. 2A). Resistance of HIV-1 isolates to ddI and d4T has been mapped to single base pair changes flanking amino acids 74 and 75 (4, 5, 7), and some degree of cross-resistance between these two drugs has been noted in clinical settings. PERV and MLV are identical in this region but have several residues that differ from HIV-1, most notably at position 75, where the amino acid change from valine to threonine has been associated with resistance. In PERV and MLV, the amino acid at this position is glutamic acid, and the resistance of PERV and MLV to d4T could be explained by structural changes in this region that alter the binding of the nucleotide analog. Both PERV and MLV contain the Leu-to-Val mutation at position 74 associated with ddI in HIV-1 but remain sensitive to the drug, suggesting that other changes between PERV or MLV and HIV in this region compensate for the presence of the L74V mutation identified in ddI RNA. The 3TC mutation M184V is
also associated with some increase in resistance to ddI in HIV-1 (5); it should be noted that both the L74V and the M184V mutations are associated with only modest increases in ddI resistance (five- to eightfold). Although PERV and MLV are identical in this region, they appear to differ by severalfold in their sensitivity to ddI, further supporting the idea that other changes outside this region affect sensitivity. The overall homology between PERV and MLV is 60 to 70%. These results show that while sequence comparisons are useful in predicting sensitivity to antiretroviral drugs, in vitro testing provides important information as well, particularly when sequences that are not well conserved are being examined.

In spite of the considerable divergence of protease sequences between MLV and HIV-1, our previous study showed that unlike PERV, MLV was inhibited by the HIV-1 protease inhibitor indinavir (14). These results are consistent with the fact that resistance to protease inhibitors often involves multiple base pair changes, making it difficult to predict the efficacy of HIV-1 protease inhibitors against other retroviruses strictly on the basis of sequence.

These results demonstrate that PERV replication can be controlled using standard antiretroviral therapies but that, like MLV, the virus is inherently resistant to some commonly used therapies.

We thank M. Stefanis for preparation of PERV supernatant, M. Kaloss and S. Brazinski for analysis of indinavir control samples and for performing sequence comparisons, and I. Mychkovsky, L.-P. Li, and I. Burinskiy for technical assistance. We are grateful to Merck and Co. for generously supplying indinavir.

REFERENCES


