Visna in sheep as a model for chemotherapy of lentiviral central nervous system infections

Clin Microbiol Infect 1998; 4: 618–621

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INTRODUCTION

Visna virus is the prototype of the lentivirus subgroup of retroviruses. Lentivirus means slow virus; this refers to the fact that visna virus causes a slow infection in sheep which leads either to the lung disease maedi or more rarely to the central nervous system (CNS) disease visna.

Maedi, meaning shortness of breath, is a slow progressive lymphoid interstitial pneumonia which was first described in South Africa and in Montana in the USA 80 years ago. Since then it has been found to be endemic in many countries in Europe and Asia [1]. Visna, which is a slowly progressing leukoencephalomyelitis, is rarely seen even in areas where the lung disease is prevalent. Thus, in a flock of sheep with endemic slow progressive pneumonia in the Rocky Mountains, where 76% of the sheep were affected by the lung disease, only 7% showed lesions in the CNS [2].

Maedi was brought to Iceland with sheep imported from Germany in 1933 and caused a widespread epidemic in Icelandic sheep in the following two decades. In contrast to the experience in other countries, a large number of cases with CNS disease were seen in one area of Iceland where maedi was prevalent. The CNS disease was named visna, which means wasting. In Icelandic sheep, visna was characterized by loss of motor function, particularly in the hind limbs, which progressed to paralysis and ended in death. It is still not known what caused this difference in the neuropathogenicity of the infection in Iceland compared with other countries, and whether it was determined by host or viral factors, although the latter is considered more likely.

Visna virus was isolated in Iceland in 1957 in tissue culture from an infected sheep brain. In the following year, the same virus was isolated from lungs of sheep with maedi, but the lung isolates were slightly different from the brain isolates. From these and other observations it was concluded that visna and maedi are caused by the same virus and that visna is a neurologic manifestation of the more common lung disease [3]. Visna virus is therefore often referred to as maedi/visna virus (MVV). Further characterization of the virus showed it to resemble avian and murine leukemia viruses, and shortly after the demonstration of reverse transcriptase (RT) in the virions of these viruses, RT was also found in visna virions [4]. The molecular biology of visna virus and its virus–host interactions have since then been thoroughly studied in Iceland and in several laboratories both in Europe and the USA. All of these studies demonstrated that visna virus, and the related viruses CAEV (caprine arthritis encephalitis virus) and EIAV (equine infectious anemia virus), are non-oncogenic retroviruses which were classified as the lentivirus subgroup of retroviruses [5].

When the human immunodeficiency virus (HIV) was isolated in 1983 it soon became apparent that it fitted into the lentivirus subgroup [6]. Studies of visna virus thus gained increased importance because of its similarity to HIV. There is a considerable homology in the genomes of the two viruses, particularly in the *pol* genes but also in the *gag* genes. The gene organization is similar and the viruses share at least three non-structural regulatory genes with apparently identical functions. The replication cycle of visna virus is functionally very similar to that of HIV, and both viruses infect monocytes and macrophages. However, they use different cell receptors and visna virus does not

infect T-helper cells or cause noticeable immunodeficiency. In the infected host, both viruses show an acute phase early in the infection followed by a prolonged period of clinical latency, sometimes lasting for several years. When clinical signs appear, they are progressive and always lead to death. HIV and visna virus infections cause similar pathologic changes in the lungs and in the CNS, at least early in the disease, and the immune response is not effective in clearing the infection [7,8].

EFFECT OF ANTI-HIV COMPOUNDS ON VISNA VIRUS REPLICATION IN CELL CULTURE

Compounds which are active against HIV and other lentiviruses of primates have been thoroughly studied. In contrast, few studies have been done on compounds that are active against visna virus and related lentiviruses of ungulates. However, it is of interest to compare the drug sensitivities of the primate and ungulate lentiviruses to further characterize their molecular and biological relationships. Also, compounds that strongly affect the replication of both subgroups of lentiviruses may be considered to be broad-spectrum lentivirus inhibitors. Finally, because of the similar neuropathic effects of visna virus and HIV, the visna disease in sheep might be used as an animal model for in vivo testing of candidate anti-HIV drugs, particularly with regard to their potential efficacy in the treatment of HIV infections of the brain.

Experiments were therefore done to test the effect of various anti-HIV drugs on the replication of visna virus in sheep choroid plexus (SCP) cell culture [9,10]. Sulfated polysaccharides (i.e. dextran sulfate, pentosan polysulfate and heparin) and various plant lectins were found to be 10–40-fold less active against visna virus than HIV under the experimental conditions used. Since both types of compounds are known to inhibit viral adsorption and/or fusion to host cell membranes, the results may reflect differences in the surface proteins of the viruses. TIBO derivatives, which are non-nucleoside RT inhibitors with a high specificity for HIV-1, had no inhibitory effect on visna virus at subtoxic concentrations. The same was true for bicyclam derivatives, which are also highly HIV-specific compounds, supposedly affecting the fusion of the virus [11]. In contrast, certain nucleoside analogs which are inhibitors of HIV RT were found to be very active against visna virus replication. This was particularly true for 2′,3′-dideoxynucleoside (ddN) and acyclic nucleoside phosphonate (ANP) analogs [9,10]. Of the ddN analogs, dideoxycytidine (ddC) and dideoxyinosine (ddI) were the most active, whereas 9-(2-phosphonomethoxyethyl)adenine (PMEA) and (*R*)-9-(2-phosphonomethoxypropyl)adenine (PMPA) were among the most active ANP analogs. Both of these compounds are also highly active against HIV [11]. A summary of these results is shown in Table 1.

EFFECT OF PMEA ON VISNA VIRUS INFECTION IN LAMBS

In order to investigate the feasibility of using visna in sheep as an animal model for testing the in vivo efficacy of anti-HIV compounds, it was decided to study the effect of the prototype ANP analog PMEA on the early stages of visna virus infection in young lambs. PMEA had previously been tested against several other retrovirus infections in experimental animals by administration of the drug simultaneously with or slightly before inoculation with virus [12]. For the sake of comparison, a similar protocol was used in the visna virus experiment [13]. In this experiment, 2-week-old Icelandic lambs were inoculated intracerebrally with $10^{6.3}$ $TCID_{50}$ (50% tissue-culture infective dose) of visna virus KV1772, which is a biological clone with increased neurovirulence. Two groups of four lambs each received PMEA subcutaneously 1 h before intracerebral inoculation, at 10 and 25 mg/kg, respectively, and the drug treatment was continued for 6 weeks, with subcutaneous injections three times a week. A group of four lambs was inoculated in the same way but not treated with PMEA. The lambs were bled at regular intervals and the leukocytes tested for the presence of visna virus. At 7 weeks after infection, the animals were killed, and cerebrospinal fluid (CSF) and samples of tissue from various organs were collected for isolation of virus and for histopathologic examination. Table 2 summarizes the results of this experiment [13].

The PMEA treatment had an inhibitory effect on the visna virus infection, which was similar for both doses of the drug. Thus, the frequency of virus isolations was much lower in PMEA-treated than in untreated lambs. This difference was particularly

Table 1 A summary of the inhibitory effects of anti-HIV compounds on visna virus replication in SCP cell cultures

Compound	Anti-visna virus effect
Sulfated polysaccharides	(+)
Plant lectins	(+)
TIBO derivatives	−
Bicyclam derivatives	−
Dideoxynucleoside analogs	+
Acyclic nucleoside phosphonates	+

+, compound equally active against visna virus and HIV;
(+), compound less active against visna virus than against HIV;
−, compound not effective or with a negligible effect on visna virus.

Table 2 Effect of PMEA treatment on visna virus infection in lambs

Group[b]	Frequency of virus isolations[a]			Brain lesions, average grade[e]
	PBMC[c]	Brain	Other organs[d]	
I	16/24 (67%)	13/18 (72%)	15/16 (94%)	3.0
II	1/24 (4%)	2/19 (11%)	7/14 (50%)	0.5
III	0/24 (0%)	5/20 (25%)	7/14 (50%)	0.375

[a] The results are expressed as no. positive/no. tested and per cent positive (in parentheses).
[b] The lambs were either untreated (group I) or injected subcutaneously with PMEA at 10 mg/kg (group II) or 25 mg/kg (group III) three times weekly for 6 weeks.
[c] Peripheral blood mononuclear cells.
[d] Lungs, spleen, cervical lymph node, mediastinal lymph node.
[e] The severity of inflammatory brain lesions is graded on a scale of 0–6.

pronounced in the peripheral blood mononuclear cells (PBMCs) and in brain tissue. Furthermore, inflammatory lesions in the brain were much less severe and the cell counts in the CSF and the serum antibody titers against visna virus were much lower (data not shown) in the treated lambs than in the untreated controls.

The inhibitory effect of PMEA on visna virus infection in lambs is in agreement with previous studies of this anti-HIV compound in other animal models, where treatment began 1–48 h before virus inoculation [12]. The efficacy of PMEA treatment in reducing early inflammation in the brain of visna virus-infected lambs is particularly noticeable. This correlates with a considerably lower viral activity in the brains of treated compared with untreated animals.

A pharmacokinetic study of PMEA in the blood of the two groups of lambs, receiving a dose of 25 or 10 mg/kg, showed half-lives of the drug of 75 and 64 min, respectively. The drug caused no noticeable side effects during the 6 weeks of treatment.

Because of this marked prophylactic effect of PMEA treatment on visna virus infection in lambs and its safety during an extended treatment period, it was of interest to study the possible therapeutic effect of such treatment in later phases of the infection, when inflammatory brain lesions had already appeared. A number of young lambs were therefore inoculated with visna virus as in the first experiment, but treatment with PMEA was not started until 1 month later. The treatment was continued for 8 months, with three subcutaneous injections a week. The dose was 10 mg/kg during the first 6 months, but was increased to 25 mg/kg for the final 2 months of the treatment. The sheep were then killed and samples taken from the brain, lungs, spleen and lymph nodes for virus isolation. The results of this experiment [14] show that the PMEA treatment had much less effect than in the previous experiment, where treatment was begun 1 h before inoculation. Although there was a significant reduction in the number of virus isolations from the PBMCs of PMEA-treated lambs compared with untreated lambs ($P<0.05$), there was no difference in the number of virus isolations from brains and other organs collected at sacrifice, 8 months after beginning treatment, and there was no difference in CSF cell counts. Thus, the PMEA treatment which was started 4 weeks after inoculation with visna virus had only a minimal therapeutic effect on the virus infection in lambs. These results are in agreement with a recent study on the therapeutic effect of PMEA in cats with chronic feline immunodeficiency virus infection [15]. In this study, no detectable reduction was found in the viral load in plasma after subcutaneous injection of 20 mg/kg of PMEA three times a week for 6 weeks [15]. On the other hand, in a study on the efficacy of PMEA treatment against chronic simian immunodeficiency virus infection in macaques, the drug caused a marked reduction in viral load in the blood and the number of viral isolations from PBMCs [16]. However, although the drug was administered subcutaneously at a dose of 20 mg/kg daily for 4–8 weeks, it had no effect on the tissue distribution of the virus tested at various times after the end of PMEA treatment.

SUMMARY AND CONCLUSIONS

Visna virus is a lentivirus of sheep which shows molecular and biological similarities to HIV. Nucleoside analogs which are inhibitors of HIV RT were found to be highly effective inhibitors of visna virus replication in vitro. The acyclic nucleoside phosphonate analog PMEA was tested against visna virus infection in lambs. The drug was very effective against the brain infection if administered 1 h before intracerebral inoculation with visna virus. On the other hand, it showed little activity if treatment was begun 1 month after inoculation. This is in agreement with studies of PMEA treatment in other lentivirus infections, where a comparable dose was used. Visna virus infection in lambs therefore seems to be a useful animal model to study the effect of drug treatment on lentivirus infections of the brain, particularly in short-term experiments.

Acknowledgments

This work was supported by grants from the Icelandic Council of Science and the Research Fund of the University of Iceland. Support was also received from grants from the Biomedical Research Programme of the European Commission, the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek, the Belgian

Nationaal Fonds voor Wetenschappelijk Onderzoek, and the Belgian Geconcerteerde Onderzoeksacties.

References

1. Pálsson PA. In Kimberlin RH, ed. Slow virus diseases of animals and man. Amsterdam: North Holland, 1976: 17–43.
2. DeMartini JC, Brodie SJ, Concha-Bermejillo A, Lairmore MD. The pathogenesis of lymphoid interstitial pneumonia in natural and experimental ovine lentivirus infection. Clin Infect Dis 1993; 17(suppl 1): S236–42.
3. Thormar H. In Gajdusek DC, Gibbs CJ Jr, Alpers M, eds. Slow, latent and temperate virus infections. Physical, chemical and biological properties of visna virus and its relationship to other animal viruses. National Institute of Neurological Diseases and Blindness, Monograph no. 2. Washington DC: US Public Health Service, 1965: 335–40.
4. Lin FH, Thormar H. Ribonucleic acid-dependent deoxyribonucleic acid polymerase in visna virus. J Virol 1972; 6: 702–4.
5. Haase AT. Pathogenesis of lentivirus infection. Nature 1986; 322: 130–6.
6. Gonda MA, Wong-Staal F, Gallo RC, Clements JE, Narayan O, Gilden RV. Sequence homology and morphologic similarity of HTLV-III and visna virus, a pathogenic lentivirus. Science 1985; 227: 173–7.
7. Georgsson G, Pálsson PA, Pétursson G. In Racz P, Haase AT, Gluckman SC, eds. Some comparative aspects of visna and AIDS. Modern pathology of AIDS and other retroviral infections: application of contemporary methods. Basel: Karger, 1990: 82–98.
8. Gonda MA. Molecular biology and virus–host interactions of lentiviruses. Ann NY Acad Sci 1994; 724: 22–42.
9. Thormar H, Balzarini J, Holy A, et al. Inhibition of visna virus replication by 2′,3′-dideoxynucleosides and acyclic nucleoside phosphonate analogs. Antimicrob Agents Chemother 1993; 37: 2540–4.
10. Thormar H, Balzarini J, Debyser Z, Witvrouw M, Desmyter J, De Clercq E. Inhibition of visna virus replication and cytopathic effect in sheep choroid plexas cell cultures by selected anti-HIV agents. Antiviral Res 1995; 27: 49–57.
11. De Clercq E. Antiviral therapy for human immunodeficiency virus infections. Clin Microbiol Rev 1995; 8: 200–39.
12. Balzarini J, De Clercq E. Acyclic purine nucleoside phosphonates as retrovirus inhibitors. In Jeffries DJ, De Clercq E, eds. Antiviral chemotherapy. Chichester: Wiley, 1995: 41–63.
13. Thormar H, Georgsson G, Pálsson PA, et al. Inhibitory effect of 9-(2-phosphonylmethoxyethyl)adenine on visna virus infection in lambs: a model for in vitro testing of candidate anti-human immunodeficiency virus drugs. Proc Natl Acad Sci USA 1995; 92: 3283–7.
14. Thormar H, Georgsson G, Gunnarsson E, et al. Treatment of visna virus infection in lambs with the acyclic nucleoside phosphonate analogue 9-(2-phosphonylmethoxyethyl)adenine (PMEA). Antiviral Chem Chemother 1998; 9: 245–52.
15. Vahlenkamp TW, De Ronde A, Balzarini J, et al. (*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine is a potent inhibitor of feline immunodeficiency virus infection. Antimicrob Agents Chemother 1995; 39: 746–9.
16. Tsai C-C, Follis KE, Sabo A, Grant R, Bischofberger N. Efficacy of 9-(-2-phosphonylmethoxyethyl)adenine (PMEA) treatment against chronic simian immunodeficiency virus infection in macaques. J Infect Dis 1995; 171: 1338–41.