ELSEVIER

Antiviral Research 28 (1995) 359–368

Antiviral Research

Inhibition of neurotropic mouse retrovirus replication in glial cells by synthetic oligo(2′-O-methyl)ribonucleoside phosphorothioates

Sayaka Takase-Yoden [a,*], Susumu Shibahara [b], Hirokazu Morisawa [b], Rihito Watanabe [a]

[a] *Institute of Life Science, Soka University, Tangi-cho 1-236, Hachioji, Tokyo 192, Japan*
[b] *Central Research Laboratories, Ajinomoto Co. Inc., Suzuku-cho, Kawasaki 210, Japan*

Received 27 February 1995; accepted 26 July 1995

Abstract

Synthetic oligo(2′-O-methyl)ribonucleoside phosphorothioate, FS-25, which is complementary to the splicing acceptor site of neurotropic mouse retrovirus (FrC6 virus), and non-complementary analogs including 2′-O-methylinosine homo oligomer (MIS-25), both inhibited viral infection in glial cells. In addition, FS-25 and MIS-25 partially suppressed viral production of glial cells persistently infected with FrC6 virus. Both FS-25 and MIS-25 potently inhibited reverse transcriptase activity of the FrC6 virus in a cell-free system. Addition of these compounds before or after second-round infection of the FrC6 virus inhibited the accumulation of unintegrated viral DNA. These results indicate that these compounds fundamentally inhibit retrovirus production in glial cells in the same manner in which they inhibit HIV production, by blocking several viral replication pathways including fresh infection, second-round infection, and reverse transcription of the viral genome. Our novel neurotropic retrovirus is a useful experimental model for the development of drugs against HIV infection.

Keywords: Oligo(2′-O-methyl)ribonucleoside phosphorothioate; Neurotropic mouse retrovirus; Glial cell

* Corresponding author. Fax: +81 (426) 919315. E-mail: takase@sccl.t.soka.ac.jp.

SSDI 0166-3542(95)00061-5

1. Introduction

Synthetic oligonucleotides have been reported to exert inhibitory activity in vitro against several viruses including human immunodeficiency virus (HIV), herpes simplex virus, cytomegalovirus, murine leukemia virus, vesicular stomatitis virus, and papilloma virus (Kara et al., 1983; Lemaitre et al., 1987; Matsukura et al., 1987, 1989; Agrawal et al., 1988; Draper et al., 1990; Gao et al., 1990a; Hoke et al., 1991; Crooke et al., 1992; Lavignon et al., 1992; Lisziewicz et al., 1992; Ropert et al., 1992; Azad et al., 1993; Cowsert et al., 1993). One of the major problems encountered when using naturally occurring phosphodiester oligonucleotides is their rapid degradation caused by various nuclease activities in cells or culture medium (Wickstrom et al., 1986; Cazenave et al., 1987). The phosphodiester backbone has since been modified to generate oligonucleotides with greater resistance to nuclease activity. The resulting phosphorothioate oligonucleotides demonstrate increased resistance to both exo- and endonucleases (Stein et al., 1988).

Oligo(2′-O-methyl)ribonucleoside phosphorothioates are resistant to several nucleases (Dunlap et al., 1971; Hoke et al., 1991) and form a stable heteroduplex with complementary RNA (Inoue et al., 1987). These compounds exhibit size-dependent inhibition of HIV infections, with 25 mer showing the most potent inhibitory activity (Shibahara et al., 1989). These compounds must be tested on experimental animals infected with retrovirus in order to further evaluate antiviral activity. We have isolated neurotropic retrovirus from Friend murine leukemia virus (FrC6 virus). This virus proliferates in the central nervous system and glial cell lines of rats, inducing spongiform encephalopathy in the brain and reducing helper T-cell activity (Takase-Yoden and Watanabe, 1994; Watanabe and Takase-Yoden, 1995). Despite the tendency of lesions to form in the gray matter of the central nervous system, viral antigens were not detected in neuronal cells (Ikeda et al., 1995). Similarly, in human encephalopathy induced by HTLV-1 or HIV, viral infection cannot be detected in the neuronal cells, so the pathogenesis of brain damage remains unclear (Elder and Sever, 1988). Therefore, it is particularly important to study the mechanisms by which encephalopathy is induced by retrovirus infection, as well as the efficiency of treatment using antiviral agents.

2. Materials and methods

2.1. Virus and cells

Replication-competent neurotropic mouse retrovirus (FrC6 virus) was isolated by adaptation to the rat brain and rat glial cell line (C6) as previously described (Takase-Yoden and Watanabe, 1994; Watanabe and Takase-Yoden, 1995). Persistently infected rat glial cell line (FrC6-GT) contains only the replication-competent murine retrovirus genome and constantly produces the virus at a high titer. C6 cells and FrC6-GT cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

2.2. Compounds

Synthetic oligo(2′-O-methyl)ribonucleoside phosphorothioates were synthesized as previously described (Shibahara et al., 1989). FS-25 (25 mer) is complementary to the splice acceptor site of neurotropic murine leukemia virus (FrC6 virus) (3′-GAGU-AAAUGUCCGUGAGAUGGACCA-5′). MIS-25 (25-mer) is the homo oligomer of 2′-O-methylinosine phosphorothioates. 3′-Azido-3′-deoxythymidine (AZT) was purchased from Nakalai Tesque (Japan). The addition of 3 μM AZT before cell-free virus infection eliminated all detectable viral production without causing cell damage.

2.3. Assay for antiviral activity

Serial dilution of FS-25 or MIS-25 and 5×10^3 XC-PFU of FrC6 virus was inoculated to 1×10^4 of rat glial cell line (C6) on a 48-well plate. After incubating for 1 h at 37°C, the virus was removed and media containing each concentration of the compound were added. After 2 days, the compound was removed, cells were washed in the medium twice, and fresh medium was added. Reverse transcriptase activity of the virus during 5 h was measured as previously described (Takase-Yoden and Watanabe, 1994). C6 cells persistently infected with the FrC6 virus (FrC6-GT cells) were established by co-cultivation with a virus-producing T-cell line (Fle-ly) (Watanabe and Takase-Yoden, 1995). The cells were treated with the serially diluted compound for 24 h, following which the compound was removed and fresh medium was added. After 5 h, viral production was measured in terms of reverse transcriptase activity. FrC6-GT cells and C6 cells freshly infected with the FrC6 virus showed no cytopathic effect.

2.4. Detection of viral mRNA by Northern blot hybridization

FrC6-GT cells were treated with several concentrations of FS-25 or MIS-25 for 2 days, and then total cellular RNA was extracted by the acid guanidium–phenol–chloroform method (Chomczynski and Sacchi, 1987). RNA samples (20 μg) were electrophoresed through a formaldehyde gel (Maniatis et al., 1982), transferred to a membrane, and hybridized to ^{32}P-labeled Friend leukemia virus *env* DNA and ^{32}P-labeled β-actin or tublin DNA. Radioactivities in bands were measured with a BAS 2000 Bio-image Analyzer (Fuji Photo Film) as previously described (Takase-Yoden and Watanabe, 1994). The amount of viral mRNA was normalized with the amount of β-actin or tublin mRNA to allow for comparison.

2.5. Detection of unintegrated viral DNA by Southern blot hybridization

FS-25, MIS-25, or 3′-azido-3′-deoxythymidine (AZT) was added to C6 cells at 15 or 36 h postinfection with FrC6 virus at an m.o.i. of 1. At 72 h postinfection, low molecular weight DNA was prepared by differential salt precipitation (Hirt, 1976). The FrC6 genome contains a single *Eco*RI restriction endonuclease site at 3.2-kb distance from the 5′-end. Thus, *Eco*RI-digested extrachromosomal DNA equivalent to that from

3×10^5 cells was electrophoresed through a 0.8% agarose gel, transferred to a membrane, and hybridized to ^{32}P-labeled Friend leukemia virus *env* DNA. Radioactivities in bands of viral mRNA were detected by a BAS 2000 Bio-image Analyzer (Fuji Photo Film).

2.6. Assay for anti-reverse transcriptase activity in a cell-free system

Using poly(rA)p(dT)$_{12-18}$ reverse transcriptase activities of FrC6 virus were measured in solutions containing serial dilutions of FS-25 or MIS-25 as previously described (Takase-Yoden and Watanabe, 1994).

3. Results

3.1. Synthetic 2'-O-methyl-RNA phosphorothioates inhibition of mouse retrovirus production in glial cells

FS-25 is complementary to the splicing acceptor site of the neurotropic murine leukemia (FrC6) virus (3'-GAGUAAAUGUCCGUGAGAUGGACCA-5'). MIS-25 is 25 mer of 2'-O-methylinosine phosphorothioates. These compounds were tested for inhibition of FrC6 virus replication in rat glial cell line (C6). Sixteen μM of FS-25 and 4 μM of MIS-25 completely inhibited viral production when added to the culture medium prior to infection (Fig. 1A, B). FS-25 required a dosage 4 times higher than that of MIS-25 to induce more than 90% inhibition of viral production. Sixteen μM of FS-25

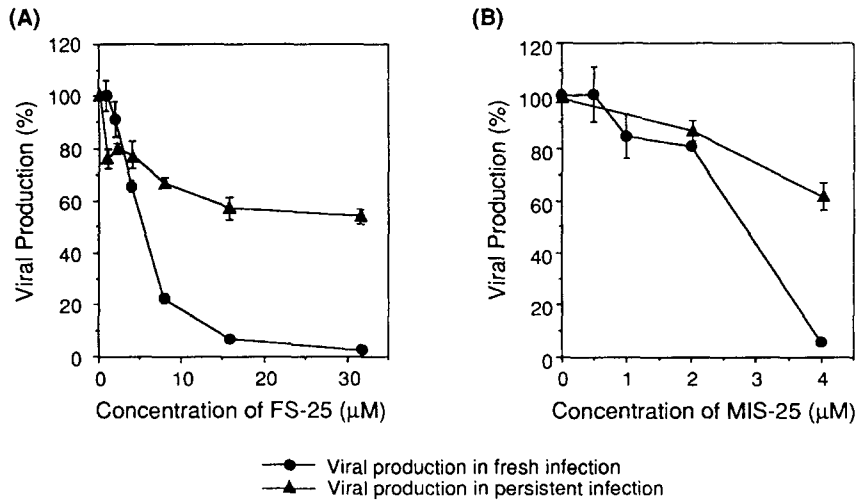

Fig. 1. Inhibitory activity of FS-25 (A) and MIS-25 (B) against viral production in fresh and persistent infection. Viral production from virus-infected cells treated with several concentrations was measured in terms of reverse transcriptase activity. Reverse transcriptase activity in supernatant of virus-infected cells in absence of compound is taken as 100%. Means and S.E.M. ($n = 3$, except for values of viral production in fresh infection in presence of MIS-25; $n = 2$) are indicated. Where bar is not seen, S.E.M. is less than range of point.

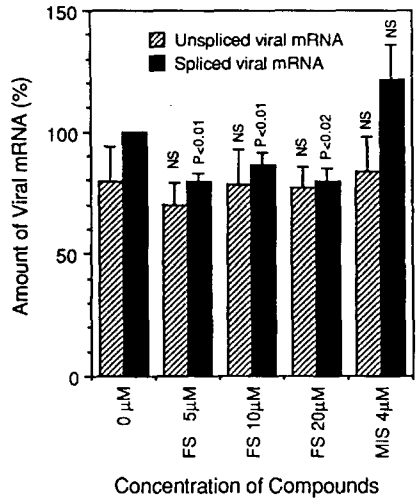

Fig. 2. Effects of FS-25 and MIS-25 on viral mRNA in persistently infected cells. FrC6-GT cells were treated with several concentrations of compounds. Amounts of viral mRNA were measured by Northern blot hybridization using 20 μg of total cellular RNA and normalized with amount of β-actin or tubulin mRNA for comparison (see Materials and methods). Amount of spliced viral mRNA in the absence of compound is taken as 100%. Means and S.E.M. ($n = 3$) are indicated. P-values indicate statistical significance at each concentration level compared to amount of spliced or unspliced viral mRNA in the absence of compound. NS, not significant.

and 4 μM of MIS-25 suppressed viral production of persistently infected and FrC6 virus-producing C6 cells to 56 and 62% of its maximum level, respectively (Fig. 1A, B). Exposure of FS-25 or MIS-25 to C6 cells for 24 h did not affect the viability of C6 cells at the concentrations investigated (data not shown).

3.2. Effects of FS-25 and MIS-25 on viral mRNA in persistent infection

We examined the effects of FS-25 on the quantity of spliced and unspliced viral mRNA in persistently infected cells. The amount of viral mRNA was normalized against the amount of β-actin or tublin mRNA in order to allow comparison. Although the quantity of unspliced viral mRNA in persistently infected cells treated with varying dosages of FS-25 or MIS-25 was unaffected, FS-25 induced a slight reduction in the level of viral spliced mRNA (Fig. 2).

3.3. Effects of FS-25 and MIS-25 on accumulation of unintegrated viral DNA in glial cells

We examined the effect of FS-25 and MIS-25 on second-round infection of the virus. Unintegrated viral DNA has been shown to accumulate in infected cells mainly due to second-round infection (Temin, 1988; Takase-Yoden and Watanabe, 1994). Therefore, accumulation of unintegrated viral DNA in infected cells in the presence of FS-25 (20

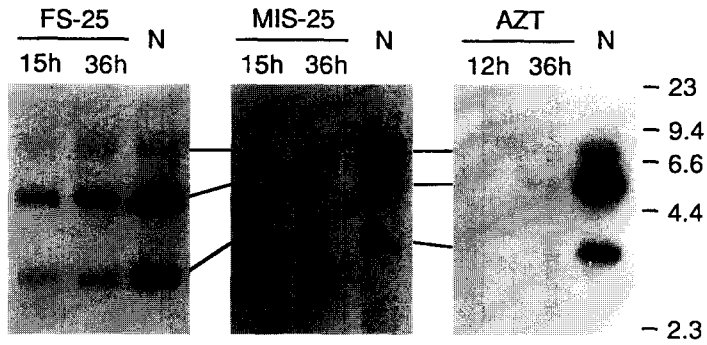

Fig. 3. Effects of FS-25 and MIS-25 on accumulation of unintegrated viral DNA. FS-25 (20 μM), MIS-25 (4 μM) and AZT (3 μM) were added at 12–15 or 36 h postinfection. N, in absence of compound. At 72 h postinfection, unintegrated viral DNA was detected by Southern blot hybridization as described in Materials and methods.

μM) and MIS-25 (4 μM) was measured. Addition of FS-25 or MIS-25 at 15 or 36 h after infection inhibited accumulation of unintegrated viral DNA (Fig. 3). Addition of 3 μM of AZT at 12 or 36 h after infection potently suppressed accumulation of unintegrated viral DNA.

3.4. Effects of FS-25 and MIS-25 on reverse transcriptase activity in vitro

Inhibitory activity of synthetic oligo(2′-O-methyl)ribonucleoside phosphorothioates against reverse transcriptase activity of the FrC6 virus was tested in a cell-free system. MIS-25 or FS-25 (both at 0.7 μM) suppressed reverse transcriptase activity of FrC6 virus to 50% of the maximum level as measured by poly(rA)p$(dT)_{12-18}$ (Fig. 4).

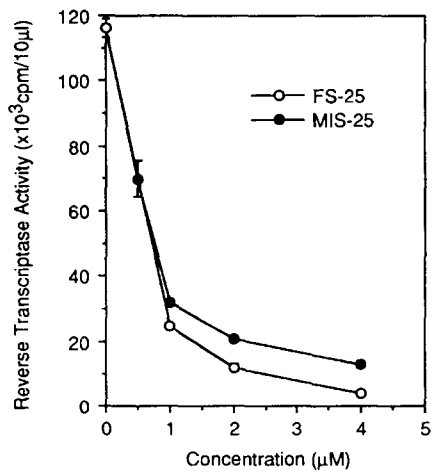

Fig. 4. Effects of FS-25 and MIS-25 on reverse transcriptase activity in cell-free system. Assay was performed as described in Materials and methods. Means and S.E.M. ($n = 3$) are indicated. Where bar is not seen, S.E.M. is less than range of point.

4. Discussion

FS-25, the synthetic oligo(2′-O-methyl)ribonucleoside phosphorothioate complementary to the splicing acceptor site of neurotropic FrC6 retrovirus, and non-complementary inosine homo oligomer (MIS-25) inhibited production of the FrC6 virus in glial cells. In fresh infections, 16 μM of FS-25 or 4 μM of MIS-25 completely inhibited viral production. These concentrations were identical to those at which these compounds inhibited HIV production (Shibahara et al., 1989). In persistent infection by the FrC6 virus, 16 μM of FS-25 and 4 μM of MIS-25 suppressed viral production to 56 and 62% of its maximum level, respectively. Inhibitory activity of FS-25 and MIS-25 against viral production in fresh infections was stronger than in persistent infection, suggesting that these compounds more effectively influence the early phase of viral infection. Antiviral activities of non-complementary phosphorothioates has previously been reported for HIV (Matsukura et al., 1987; Agrawal et al., 1988; Lisziewicz et al., 1992, 1993), herpes simplex virus (Hoke et al., 1991), and cytomegalovirus (Azad et al., 1993). In the case of oligodeoxynucleoside phosphorothioates, inhibitory activity against the early phase of HIV (Lisziewicz et al., 1992) and cytomegalovirus (Azad et al., 1993) infection was reported. The fact that MIS-25 inhibited the production of both the HIV and mouse retrovirus suggests that a common inhibitory mechanism may be involved. In the case of the herpes simplex virus, non-complementary phosphorothioates probably directly inhibit viral DNA polymerase or interact with viral particles (Gao et al., 1989, 1990a,b). Furthermore, it has been shown that phosphorothioates directly bind to the reverse transcriptase molecule of avian myeloblastosis virus through sulfur contained in the compounds (Hatta et al., 1993). These reports indicate that phosphorothioates exhibit inhibition against viral replication by interacting with components of viral particles other than the viral genome, presumably through physiological properties such as electric charge.

Previously, the specific effects of complementary phosphorothioate on HIV proliferation were not clear, and this complementary structure was shown to be even less effective than the homo oligomer in suppressing HIV production in fresh infections (Matsukura et al., 1987; Agrawal et al., 1988). In the present study, using the neurotropic mouse retrovirus, it was again shown that MIS-25, the homo oligomer, was more potent in inhibiting fresh infections of the FrC6 virus than was complementary FS-25. In persistent infection, the inhibitory effects of FS-25 and MIS-25 against viral production were almost identical. Specific effects of the FS-25 designed to block the splicing acceptor site were not demonstrated in our experiments. Northern hybridization analysis showed only slight reductions in spliced viral mRNA levels after FS-25 treatment (Fig. 2). A higher dosage of anti-sense oligonucleotides may be needed in order to suppress the splicing of viral RNA (Stein et al., 1988). Synthetic phosphorothioate complementary to the splicing acceptor site of the HIV genome does not suppress viral production in persistent HIV infections (Shibahara et al., 1989). This may be due to differences in permeability of the cell membrane, accessibility or affinity to the targeted viral components, stability in cells, which may depend on the sequence of synthetic oligomers, or amount of the targeted viral components in persistently infected cells.

Unintegrated viral DNA accumulated mainly as a result of second-round infection

(Temin, 1988). In our previous study (Takase-Yoden and Watanabe, 1994), addition of anti-FrC6 neutralizing antibody to the culture within 24 h postinfection inhibited accumulation of unintegrated viral DNA. However, addition of the antibody after 30–36 h postinfection did not inhibit accumulation of unintegrated viral DNA, indicating that second-round infection occurred within 30 h postinfection. In this experiment, administration of FS-25 or MIS-25 at 15 h postinfection before onset of second-round infection inhibited accumulation of viral DNA, indicating that these compounds inhibit second-round infection of the virus. Administration of MIS-25 or FS-25 at 36 h postinfection after onset of second-round infection suppressed accumulation of unintegrated viral DNA in the same manner as AZT. AZT inhibits accumulation of unintegrated viral DNA by blocking reverse transcriptase activity (Takase-Yoden and Watanabe, 1994). These results indicate that MIS-25 and FS-25 exhibit anti-reverse transcriptase activity in culture cells. Both of these compounds inhibited reverse transcriptase activity as measured by poly(rA)p$(dT)_{12-18}$ in a cell-free system (Fig. 4). Therefore, it is reasonable to assume that FS-25 and MIS-25 inhibited second-round infection of the virus by blocking reverse transcriptase activity.

These results suggest that FS-25 and MIS-25 inhibit viral production by blocking several viral replication pathways, similar to the inhibition of HIV infection by synthetic phosphorothioates (Shibahara et al., 1989). Our previous study using AZT and dextran sulfate demonstrated that these drugs show potent inhibitory against the FrC6 virus infection, as it does against HIV infection (Takase-Yoden and Watanabe, 1994). Many functional surface antigens reported to be expressed on lymphocytes are also found on the membranes of neurons and glial cells in the central nervous system (Williams and Gagnon, 1982). Actually, FrC6 virus infects rat T-lymphocytes in addition to central nervous cells (Watanabe and Takase-Yoden, 1995) Furthermore, FrC6 virus and its molecular clone A8 virus (Ikeda et al., 1985) infect and proliferate in the rat brain for over a 6-month term (paper in preparation). Our newly isolated virus should prove beneficial for screening drugs against HIV, because a number of cell lines have been derived from the rat central nervous system that are easily maintained at the P2 experimental level after FrC6 virus infection and the gene manipulation.

Acknowledgements

The authors wish to thank Keiko Wakabayashi for performing the assays used in this study and Sachiko Kimata for synthesizing modified oligonucleotides. This work was supported in part by a grant from the Science and Technology Agency of Japan (13073-2125-14).

References

Agrawal, S., Goodchild, J., Civeira, M.P., Thornton, A.H., Sarin, P.S. and Zamecnik, P.C. (1988) Oligodeoxynucleoside phosphoramidates and phosphorothiates. Proc. Natl. Acad. Sci. USA 85, 7079–7083.

Azad, R.F., Driver, V.B., Tanaka, K., Crooke, R.M. and Anderson, K.P. (1993) Antiviral activity of a phosphorothioate oligonucleotide complementary to RNA of the human cytomegalovirus major immediate-early region. Antimicrob. Agents Chemother. 37, 1945–1954.

Cazenave, C., Chevrier, M., Thuong, N.T. and Helene, C. (1987) Rate of degradation of [α]- and [β]-oligodeoxynucleotides in *Xenopus* oocytes. Nucleic Acid Res. 15, 10507–10502.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Cowsert, L.M., Fox, M.C., Zon, G. and Mirabelli, H.K. (1993) In vitro evaluation of phosphorothioate oligonucleotides targeted to the E2 mRNA of papillomavirus: potential treatment for genital warts. Antimicrob. Agents Chemother. 37, 171–177.

Crooke, R.M., Hoke, G.D. and Shoemaker, J.E.E. (1992) In vitro toxicological evaluation of ISIS 1082, a phosphorothioate oligonucleotide inhibitor of herpes simplex virus. Antimicrob. Agents Chemother. 36, 527–532.

Draper, K.G., Ceruzzi, M., Kmetz, M.E. and Sturzen-Dercker, L.J. (1990) Complementary oligonucleotide sequence inhibits both Vmw65 gene expression and replication of herpes simplex virus. Antiviral Res. 13, 151–164.

Dunlap, B.E., Friderici, K.H. and Rottman, F. (1971) 2′-O-methyl polynucleotides as templates for cell-free amino acid incorporation. Biochemistry 10, 2581–2587.

Elder, G.A. and Sever, J.L. (1988) AIDS and neurological disorders: an overview. Ann. Neurol. 23 (Suppl.), S4–S6.

Gao, W.-I., Stein, C.A., Cohen, J.S., Dutschman, G.E. and Cheng, Y. (1989) Effect of phosphorothioated homo-oligodeoxynucleotides on herpes simplex virus type 2-induced DNA polymerase. J. Biol. Chem. 264, 11521–11526.

Gao, W.-I., Hanes, R.N., Vazquez-Padua, M.A., Stein, C.A. and Cheng, J.S.C.Y. (1990a) Inhibition of herpes simplex virus type 2 growth by phosphorothioate oligodeoxynucleotides. Antimicrob. Agents Chemother. 34, 808–812.

Gao, W.I., Jaroszewski, J.W., Cohen, J.S. and Cheng, Y. (1990b) Mechanism of inhibition of herpes simplex virus type 2 growth by 28-mer phosphorothioate oligodeoxycytidine. J. Biol. Chem. 265, 2172–2178.

Hatta, T., Kim, S.-G., Nakashima, H., Yamamoto, N., Sakamoto, K., Yokoyama, S. and Takaku, H. (1993) Mechanisms of the inhibition of reverse transcription by unmodified and modified antisense oligonucleotides. FEBS Lett. 330, 161–164.

Hirt, B. (1967) Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. 26, 365–369.

Hoke, G.D., Draper, K., Freier, S.M., Gonzales, C., Drive, V.B., Zounes, M.C. and Ecker, D.J. (1991) Effects of phosphorothioate capping on antisense oligonucleotide stability, hybridization and antiviral efficacy versus herpes simplex virus infection. Nucleic Acids Res. 19, 5743–5748.

Ikeda, T., Takase-Yoden, S. and Watanabe, R. (1995) Characterization of monoclonal antibodies recognizing neurotropic Friend murine leukemia virus. Virus Res. 38, 297–304.

Inoue, H., Hayase, Y., Iwai, S. and Ohtsuka, E. (1987) Sequence-dependent hydrolysis of RNA using modified oligonucleotide splints and RNaseH. FEBS Lett. 215, 327–330.

Kara, J., Mach, O. and Smrt, J. (1983) Antiviral and anticellular effects of synthetic (2′-5′)-oligoadenylate (A2′p5′A2′p5′A) in Rauscher murine leukemia. Acta Virol. 27, 477–483.

Lavignon, M., Tounekti, N., Rayner, B., Imbach, J.-L., Keith, G., Paoletti, J. and Malvy, C. (1992) Inhibition of murine leukemia virus by nuclease-resistant alpha-oligonucleotides. Antisense Res. Dev. 2, 315–324.

Lemaitre, M., Bayard, B. and Lebleu, B. (1987) Specific antiviral activity of a poly(L-lysine)-conjugated oligodeoxyribonucleotide sequence complementary to vesicular stomatitis virus N protein mRNA inhibition site. Proc. Natl. Acad. Sci. USA 84, 648–652.

Lisziewicz, J., Sun, D., Lotman, M., Agrawal, S., Zamecnik, P. and Gallo, R. (1992) Specific inhibition of human immunodeficiency virus type 1 replication by antisense oligonucleotides: an in vitro model for treatment. Proc. Natl. Acad. Sci. USA 89, 11209–11213.

Lisziewicz, J., Sun, D., Metelef, V., Zamecnik, P., Gallo, R.C. and Agrawal, S. (1993) Long-term treatment of human immunodeficiency virus-infected cells with anti-sense oligonucleotide phosphorothioate. Proc. Natl. Acad. Sci. USA 90, 3860–3864.

Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, New York.

Matsukura, M., Shinozuka, K., Zon, G., Mitsuya, H., Reitz, M., Choen, J.S. and Broder, S. (1987) Phosphorothioate analogs of oligodeoxynucleotides: inhibitors of replication and cytopathic effects of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 84, 7706–7710.

Matsukura, M., Zon, G., Shinozuka, K., Robert-Guroff, M., Shimada, T., Stein, C.A., Mitsuya, H., Wong-Staal, F., Cohen, J.S. and Broder, S. (1989) Regulation of viral expression of human immunodeficiency virus in vitro by an antisense phosphorothiate oligodeoxynucleotide against rev (art/trs) in chronically infected cells. Proc. Natl. Acad. Sci. USA 86, 4244–4282.

Ropert, C., Lavignon, M., Dubernet, C., Couvreur, P. and Malvy, C. (1992) Oligonucleotides encapsulated in pH sensitive liposomes are efficient toward Friend retrovirus. Biochem. Biophys. Res. Commun. 183, 879–885.

Shibahara, S., Mukai, S., Morisawa, H., Nakashima, H., Kobayashi, S. and Yamamoto, N. (1989) Inhibition of human deficiency virus (HIV-1) replication by synthetic oligo-RNA derivatives. Nucleic Acids Res. 17, 239–252.

Stein, C.A., Subasinghe, C., Shinozuka, K. and Cohen, J.S. (1988) Physiochemical properties of phosphorothioate oligodeoxynucleotide. Nucleic Acids Res. 18, 1763–1769.

Takase-Yoden, S. and Watanabe, R. (1994) Accumulation of unintegrated viral DNA in rat glial cells infected with neurotropic Friend murine leukemia virus. Virus Res. 32, 1–12.

Temin, H.M. (1988) Mechanism of cell killing/cytopathic effects by nonhuman retroviruses. Rev. Infect. Dis. 10, 399–405.

Watanabe, R. and Takase-Yoden, S. (1995) Gene expression of neurotropic retrovirus in CNS. Progr. Brain Res. 105, 255–262.

Wickstrom, E. (1986) Oligonucleotide stability in subcellular extracts and culture media. J. Biochem. Biophys. Methods 13, 97–102.

Williams, A.F. and Gagnon, J. (1982) Neuronal cell Thy-1 glycoprotein: homology with immunoglobulin. Science 216, 696–703.