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The cooperative effect of interferon-α and ribavirin on subacute sclerosing panencephalitis (SSPE) virus infections, in vitro and in vivo

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Abstract

We studied the effects of two antiviral agents, human interferon-α (IFN-α) and ribavirin, on subacute sclerosing panencephalitis (SSPE) virus infections in hamsters. By intracranial administration, IFN-α alone improved the survival of infected hamsters by 20% at a dose of 6×10^4 IU/kg every other day for 10 days. When the dose of IFN-α was increased incrementally to 6×10^6 IU/kg, the survival rate increased by 70% in a dose-dependent manner. The combination of IFN-α and ribavirin had a synergic inhibitory effect on the replication of SSPE virus in cell culture. Combination of IFN-α (at a dose of 6×10^5 IU/kg) with ribavirin (at a dose of 1 mg/kg) completely prevented mortality. This was significantly better than either IFN-α or ribavirin monotherapy ($p < 0.05$). Under the conditions used, IFN-α did not enhance the toxicity of ribavirin in hamsters. Intraventricular administration of high dose IFN-α and ribavirin may have potential usefulness in the treatment of patients with SSPE. © 1998 Elsevier Science B.V.

Keywords: SSPE; Ribavirin; IFN-α; Combination therapy

1. Introduction

Subacute sclerosing panencephalitis (SSPE) is a rare but progressive and fatal central nervous system disorder that results from a persistent altered measles virus (SSPE virus) infection. A few compounds, such as inosiplex (Jones et al., 1982; Fukuyama et al., 1987) and amantadine (Robertson et al., 1980), have been claimed favorably affect the survival of patients with SSPE, but these compounds are by no means able to inhibit the virus replication and cure the disease. IFN-α is a broad-spectrum antiviral agent which acts

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against both DNA viruses and RNA viruses. It inhibits the replications of measles and SSPE virus strains in cell culture systems; however, the evidence of definitive efficacy in the clinic is lacking (Kuroki et al., 1989; Panitch et al., 1986; Yoshioka et al., 1989; Yalaz et al., 1992; Generoso et al., 1993; Cianchetti et al., 1994).

We examined a wide variety of antiviral compounds for their inhibitory effects on measles and SSPE virus strains in vitro and found that ribavirin inhibited the replication of various SSPE virus strains (Hosoya et al., 1989). The SSPE virus was able to replicate in the brain of hamsters and caused encephalitis which resulted in hyperirritability, myoclonus, convulsion, and then death (Sugita et al., 1984). Similar signs are observed in patients with SSPE. Ribavirin was examined for its anti-SSPE virus activity using the hamster SSPE model. Intracranial administration of ribavirin at a dose of 10 mg/kg completely prevented mortality and inhibited the replication of the SSPE virus in the brains of infected hamsters (Honda et al., 1994). But once daily intracranial administration of ribavirin at a dose of 20 mg/kg for uninfected hamsters reduced survival to 80%. The effective and safe (therapeutic) range of ribavirin for SSPE virus-infected hamsters was narrow (Ishii et al., 1996). The combination therapy of ribavirin and IFN-α is reported to be effective for the treatment of patients with hepatitis C (Schvarcz et al., 1995). In this study, we evaluated the effects of IFN-α alone and ribavirin alone, and the combination of IFN-α with ribavirin on SSPE virus infection in cell culture and hamsters.

2. Materials and methods

2.1. Virus

The virus strain used was the SSPE virus Yamagata-1 strain, which was originally isolated from the brain of a SSPE patient. A cell-free SSPE virus was prepared for in vitro assay as described previously (Hosoya et al., 1989). Briefly, to prepare cell-free SSPE virus, virus-infected cells were co-cultivated with uninfected Vero cells, scraped off by glass beads in 5 ml of a solution consisting of 0.0038 M KH_2PO_4, 0.0072 M K_2HPO_4, 0.0049 M L-glutamic acid, 0.128 M saccharose and 1.0% bovine serum albumin in water, and sonicated at 80 W for 15 s. After centrifugation at $1600 \times g$ for 10 min, the supernatant containing the cell-free SSPE virus was collected and used as the virus stock. A virus-infected cell suspension was prepared for in vivo assays, as described previously (Honda et al., 1994). Virus-infected cells were co-cultivated with uninfected Vero cells. When the cytopathic effect induced by SSPE virus was maximal, virus-infected cells were dispersed with 0.25% trypsin solution at 37°C, collected by centrifugation at $1600 \times g$ for 10 min, and resuspended in phosphate-buffered saline (PBS). The suspension contained approximately 10^6 uninfected cells and 10^4 infected cells per ml.

2.2. Compound

Ribavirin [1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide] was provided by Yamasa Shoyu, Chiba, Japan. IFN-α was provided by Sumitomo, Osaka, Japan.

2.3. Antiviral activity in cell culture

Vero cells were seeded in 1.6 cm-diameter wells of 24 tissue culture plates at 1×10^5 cells per well and incubated at 35°C. After a 2-day incubation, when the cells were grown to confluency, the cell monolayers were incubated with serial dilutions of IFN-α for 24 h. The medium was withdrawn, and to each well 30–40 plaque forming units (PFU) of SSPE virus in 0.5 ml maintenance medium were added. After a 2-h virus adsorption period at 35°C, cell cultures were washed twice with Eagle's minimum essential medium (EMEM), and combinations of IFN-α (50, 25, 12.5, 6.25, 3.13 and 0 IU/ml) with ribavirin (20, 10, 5.0, 2.5, 1.25 and 0 μg/ml) were added to the cell cultures. The cells were then incubated for 5 days at 35°C in a CO_2 incubator, and after staining with neutral red, virus plaques were counted. The concentration of compound required to inhibit the virus plaque number by 50% was estimated as the 50% inhibitory concentration (IC_{50}). Data were plotted

and analyzed by the isobologram method (Elion et al., 1954). The fractional inhibitory concentration (FIC) for each pair, i.e. compound ribavirin plus IFN-α, was calculated as follows: $FIC_{ribavirin}$ = (concentration of ribavirin in the combination at the end point)/(concentration of ribavirin alone required to achieve that end point) and FIC_{IFN} = (concentration of IFN-α in the combination at the end point)/(concentration of IFN-α alone required to achieve that end point). The combinations resulting in an additive antiviral effect ($FIC_{ribavirin} + FIC_{IFN} = 1$) are represented by straight lines (unity lines) on the isobolograms. When the combination results in synergy, i.e. a stronger antiviral effect than the sum of the individual effects ($FIC_{ribavirin} + FIC_{IFN} < 1$), the line shifts below the unity line. When the combination results in antagonistic activity ($FIC_{ribavirin} + FIC_{IFN} > 1$), the line shifts above the unity line.

2.4. Antiviral efficacy in hamster SSPE model

Golden Syrian hamsters (3 weeks old) were used for experiments. Ten hamsters were used in each treatment group. 50 μl of the virus-infected cell suspension containing approximately 500 PFU was injected 2 mm deep with a 27-gauge needle, at two 10-h intervals into the subarachnoid space of hamsters under ether anesthesia. Intracranial administration of IFN-α alone (6×10^6, 6×10^5, 6×10^4, 6×10^3, or 0 IU/kg), ribavirin alone (10, 5, 1, 0.2 or 0 mg/kg), or combination of IFN-α (6×10^5 IU/kg) with ribavirin (5, 1, 0.2, or 0 mg/kg) was started at 12 h after the initial virus inoculation and then repeated every 48 h for IFN-α and every 24 h for ribavirin for 10 days. The number of deaths caused by the disease was monitored for 56 days after infection. Each hamster's brain was removed aseptically either at death (due to the disease) or at the end of the observation period for those animals that survived. Brains were washed twice with PBS, homogenized, and suspended in EMEM. The suspensions were diluted through a series of ten-fold dilutions. 100 μl of the dilution was inoculated into duplicates of Vero cell cultures in 24-well microtiter plates. The number of typical plaques was counted after 5 days of incubation and the infectious virus titer for each brain was determined.

2.5. Toxicity for hamsters

IFN-α (6×10^5 or 0 IU/kg) and ribavirin (10, 5.0, 1.0, or 0 mg/kg) were administered to uninfected hamsters intracranially every 48 h for IFN-α and every 24 h for ribavirin for 10 days. At 56 days after the initial injection, the survival rate for each treated group was determined.

2.6. Statistical analysis

Statistical analysis to compare treatment groups was done by using the Chi squared test (for mortality data) or Student's *t* test (for virus titer data).

3. Results

3.1. Effects of IFN-α and ribavirin on virus replication in cell culture

IFN-α and ribavirin inhibited SSPE virus replication at concentrations that were significantly lower than the cytotoxic concentration. Virus replication was reduced to 50% of that of the control at an IFN-α concentration of 30 IU/ml. Ribavirin reduced virus replication by 50% at a concentration of 11.5 μg/ml, and inhibited it completely at a concentration of 50 μg/ml. When the infected cells were treated with both IFN-α and ribavirin, a synergic effect was observed. A combination of IFN-α, at a concentration of 15 IU/ml (1/2 IC_{50}), and ribavirin, at a concentration of 2 μg/ml (1/6 IC_{50}), inhibited virus replication by 50% (Fig. 1).

3.2. Effects of IFN-α and ribavirin on survival of infected hamsters

All untreated-infected hamsters and all infected hamsters treated with IFN-α at a dose of 6×10^3 IU/kg died, with a median time of death of 7.1 days and 9.6 days, respectively. When the dose of

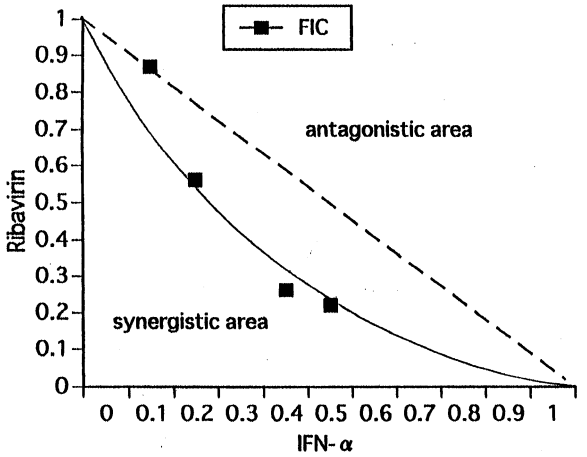

Fig. 1. Antiviral efficacy of combination of ribavirin with IFN-α against SSPE virus in vitro. $FIC_{ribavirin} + FIC_{IFN} = 0.96, 0.77, 0.62, 0.72 < 1$.

IFN-α was increased incrementally to 6×10^6 IU/kg, the survival rate increased in a dose-dependent manner. A 70% survival rate was observed at the highest dose (Fig. 2). When the dose of ribavirin was increased incrementally to 10 mg/kg, the survival rates increased in a dose-dependent manner, with 100% survival at the highest dose (Fig. 3). IFN-α was administered intracranially at a dose of 6×10^5 IU/kg (50% surviving dose), combined with ribavirin at various doses (5.0, 1.0, 0.2, or 0 mg/kg). All hamsters survived when IFN-α was combined with ribavirin at doses of 5.0 or 1.0 mg/kg. The survival rate was significantly higher than that of those treated with either ribavirin or IFN-α alone ($P < 0.05$) (Fig. 4).

3.3. Effects of IFN-α and ribavirin on virus replication in the brain of hamsters

All infected but untreated hamsters died and the infectious virus titer in the brains reached 1.7×10^5 PFU/g at death. When hamsters were treated with IFN-α at a dose of 6×10^5 IU/kg and ribavirin at a dose of 0.2 mg/kg, two hamsters died and the infectious titer was 2.5 × 104 PFU/g. No infectious virus was detected in any surviving hamsters at the end of the observation period (Table 1).

3.4. Toxicity of IFN-α and ribavirin for hamsters

IFN-α showed no toxicity in uninfected hamsters by intracranial administration at a dose of

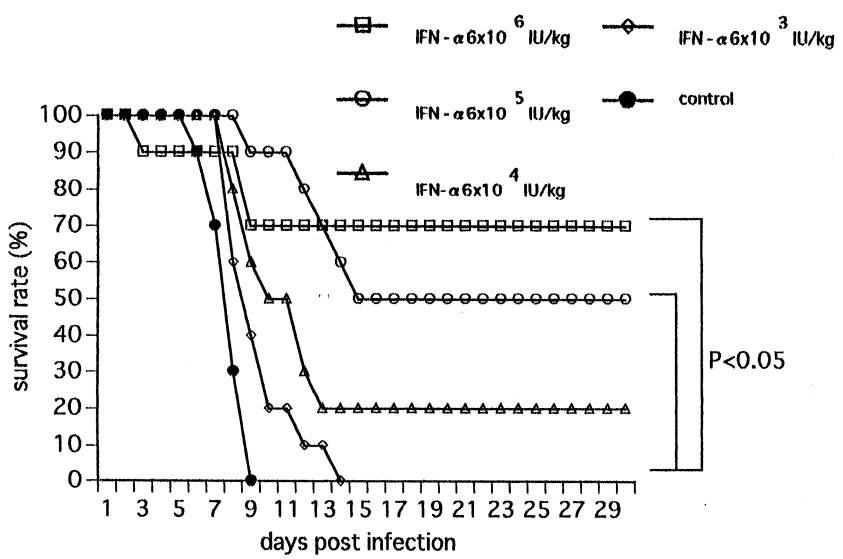

Fig. 2. Survival rate for hamsters ($n = 10$) infected with SSPE virus and treated intracranially with IFN-α at a dose of 0, 6×10^3, 6×10^4, 6×10^5, or 6×106 IU/kg every 2 days for 10 days. Intracranial administration of IFN-α at a dose of 6×10^5 and 6×10^6 IU/kg every 2 days significantly improved the survival rate to 50% ($P < 0.05$) and 70% ($P < 0.05$), respectively.

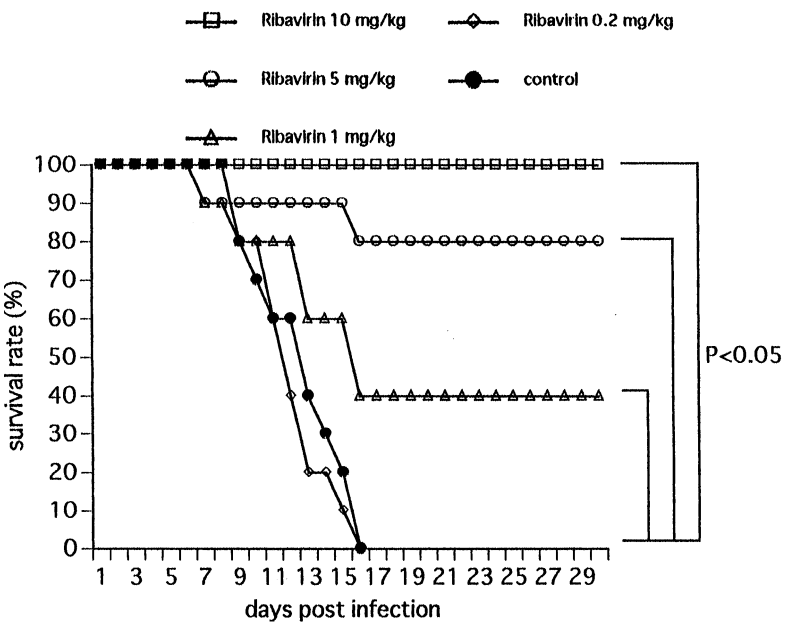

Fig. 3. Survival rate for hamsters ($n = 10$) infected with SSPE virus and treated intracranially with ribavirin at a dose of 0, 0.2, 1, 5, or 10 mg/kg per day for 10 days. Intracranial administration of ribavirin at a dose of 1, 5, and 10 mg/kg per day increased the survival rates to 40% ($P < 0.05$), 80% ($P < 0.05$), and 100% ($P < 0.05$), respectively.

6×10^5 IU/kg. Ribavirin showed toxicity in hamsters when injected intracranially at a dose of 20 mg/kg. When IFN-α was administered at a dose of 6×10^5 IU/kg, combined with ribavirin, IFN-α did not enhance the toxicity of ribavirin in hamsters (Table 2).

4. Discussion

IFN-α has a marginal effect in the clinical course of patients with SSPE when administered intraventricularly or intrathecally at a dose of 1×10^6 IU every 2 days (Panitch et al., 1986; Kuroki et al., 1989; Yoshioka et al., 1989; Yalaz et al., 1992; Generoso et al., 1993; Cianchetti et al., 1994). This corresponds approximately to a 5×10^4 IU/kg per dose. In our hamster SSPE model, only 20% of hamsters survived when IFN-α was administered at a dose of 6×10^4 IU/kg. When the dose increased to 6×10^6 IU/kg, the survival rate increased to 70%. These results suggest that patients need to be dosed with IFN-α at a high concentration to improve the clinical course and the survival rate.

Ribavirin is an antiviral agent which acts against various viruses including the SSPE virus (Hosoya et al., 1989; Shigeta et al., 1992). When ribavirin was administered to hamsters infected intracranially with SSPE virus, the symptoms of the disease decreased and the survival rate of hamsters increased in a dose-dependent manner. Ribavirin at a dose of 10 mg/kg per day resulted in 100% survival of infected hamsters; however, the minimal toxic ribavirin dose for hamsters was calculated to be 20 mg/kg (Honda et al., 1994). Thus, the therapeutic range appeared to be very narrow. Since the combination of IFN-α and ribavirin had a synergistic effect in vitro, we evaluated the effect of the combination of these two compounds in vivo. IFN-α enhanced the therapeutic effect of ribavirin on SSPE virus infection, but not the toxicity of ribavirin, in hamsters. These results suggest that the intracisternal or intraventricular administration of a safe dose of ribavirin combined with a high dose of IFN-α could be tolerated for patients with SSPE and improve the clinical course of the disease. The combination therapy of ribavirin and IFN-α may

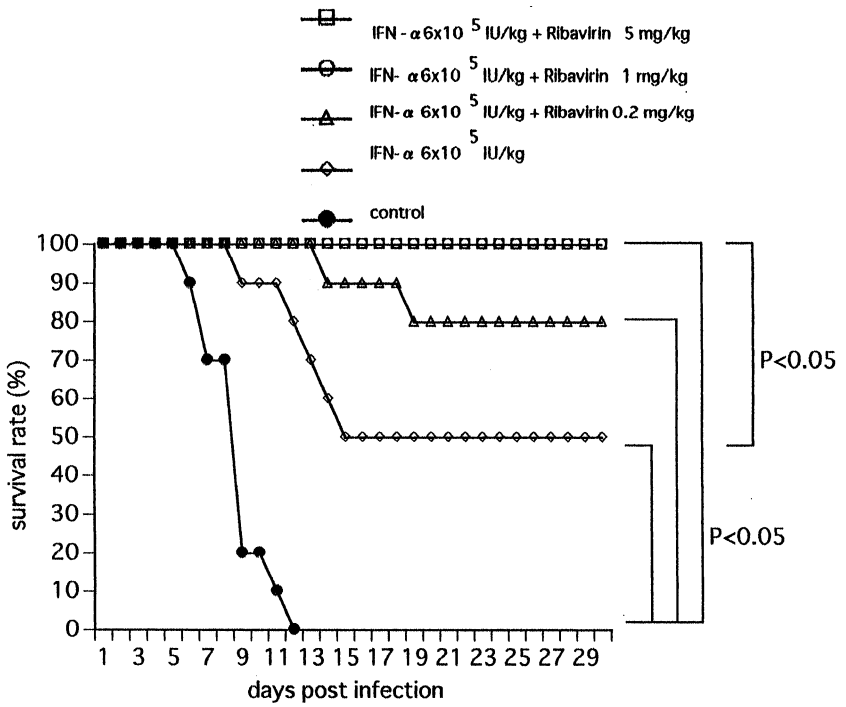

Fig. 4. Survival rate for hamsters ($n = 10$) infected with SSPE virus and treated intracranially with of IFN-α at a dose of 6×10^5 IU/kg/every 2 days combined with ribavirin at a dose of 0, 0.2, 1, or 5 mg/kg per day for 10 days. The combination of IFN-α with ribavirin at a dose of 0.2, 1 and 5 mg/kg per day significantly improved the survival rate to 80% ($P < 0.05$), 100% ($P < 0.05$) and 100% ($P < 0.05$), respectively.

also be useful for the treatment of patients with virus infections such as respiratory syncytial virus pneumonia (Wheeler et al., 1993; Englund et al., 1994) and Lassa fever, against which ribavirin has been reported to be effective.

Ribavirin, a nucleoside analogue, exerts its antiviral activity by depleting the intracellular pool of nucleotides, in particular GTP, and by blocking formation of some viral code RNA polymerases. The antiviral effects of IFN-α are mediated through induction of 2′,5′-oligo(A) synthetase, or a protein kinase, or a combination of both. In general, a synergistic effect depends on a combination of drugs with different functions cooperating with each other and this is apparently the case for the combination of IFN-α and ribavirin. In summary, the combination of IFN-α and ribavirin inhibited the in vitro and in vivo replication of SSPE virus to a greater extent than either single agent. These results indicate that

Table 1
Infectious SSPE virus titer in brains (plaque forming units/gram of brain)

	Control	Ribavirin (mg/kg) + 6×10^5 IU/kg IFN-α		
		0.2	1	5
Survival rate (%)	0 (0/10)	80 (8/10)	100 (10/10)	100 (10/10)
Virus titer	174 000 ± 82 000[a]	25 000 ± 5000	0[b]	0

[a] Infectious virus titer (mean ± S.D.) of hamsters that died from SSPE virus infection.
[b] No infectious virus was detected in any surviving hamsters.

Table 2
Survival rate of uninfected hamsters following therapy of ribavirin with IFN-α

IFN-α (IU/kg)	Survival rate (%)				
	Ribavirin (mg/kg)				
	0	1	5	10	20
0	100	100	100	100	80
6×10^5	100	100	100	100	80

further studies should be done to explore the clinical potential of this combination therapy.

References

Cianchetti, C., Fratta, A.L., Muntoni, F., Marrosu, G., Marrousu, M.G., 1994. Toxic effect of intraventricular interferon-alpha in subacute sclerosing panencephalitis. Ital. J. Neurol. Sci. 15, 153–155.

Elion, G.B., Singer, S., Hitchings, S., 1954. Antagonists of nucleic acid derivatives. Synergism in combination of biochemically related antimetabolites. J. Biol. Chem. 208, 477–488.

Englund, J.A., Piedra, P.A., Ahn, Y.M., Gilbert, B.E., Hiatt, P., 1994. High-dose, short-duration ribavirin aerosol therapy compared with standard ribavirin therapy in children with suspected respiratory syncytial virus infection. J. Pediatr. 125, 635–641.

Fukuyama, Y., Nihei, S., Matsumoto, S., Tateishi, J., Sakuma, A., 1987. Clinical effects of MND-19 (inosiplex) on subacute sclerosing panencephalitis. Brain Develop. 9, 270–283.

Generoso, G., Yamani, S., Crowell, J., Stigsby, B., Nester, M., Kanaan, I., Jallu, A., 1993. Combined oral isoprinosine-intraventricular α-interferon therapy for subacute sclerosing panencephalitis. Brain Develop. 15, 346–355.

Honda, Y., Hosoya, M., Ishii, T., Shigeta, S., Suzuki, H., 1994. Effect of ribavirin on subacute sclerosing panencephalitis virus infections in hamsters. Antimicrob. Agents Chemother. 38, 653–655.

Hosoya, M., Shigeta, S., Nakamura, K., De Clercq, E., 1989. Inhibitory effect of selectedantiviral compounds on measles (SSPE) virus replication in vitro. Antiviral Res. 12, 87–98.

Ishii, T., Hosoya, M., Mori, S., Shigeta, S., Suzuki, H., 1996. Effective ribavirin concentration in hamster brains for antiviral chemotherapy for subacute sclerosing panencephalitis. Antimicrob. Agents Chemother. 40, 241–243.

Jones, C.E., Dyken, P.R., Huttenlocher, P.R., Jabbour, J.T., Maxwell, K.W., 1982. Inosiplex therapy in subacute sclerosing panencephalitis. Lancet i, 1034–1037.

Kuroki, S., Tsutsui, T., Yoshioka, M., Mizue, H., Kita, M., Kishida, T., 1989. The effect of interferon on subacute sclerosing panencephalitis. Brain Develop. 11, 65–69.

Panitch, H.S., Plascencia, J.G., Norris, F.H., Cantell, K., Smith, R.A., 1986. Subacute sclerosing panencephalitis: remission after treatment with intraventricular interferon. Neurology 36, 562–566.

Robertson, W.C., Clark, D.B., Markesberg, W.R., 1980. Review of 38 cases of subacute sclerosing panencephalitis: effect of amantadine on the natural course of thedisease. Ann. Neurol. 8, 422–425.

Schvarcz, R., Yun, Z.B., Sonnerborg, A., Weiland, O., 1995. Combined treatment with interferon alpha-2b and ribavirin for chronic hapatitis C in patients with aprivious non-response or non-sustained response to interferon alone. J. Med. Virol. 46, 43–47.

Shigeta, S., Mori, S., Baba, M., Ito, M., Honzumi, K., Nakamura, K., Oshitani, H., Numazaki, Y., Matsuda, A., Obara, T., Shuto, S., De Clercq, E., 1992. Antiviral activities of ribavirin, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide, and 6′-(R)-6′-C-methylneplanocin A against several ortho- and paramyxoviruses. Antimicrob. Agents Chemother. 36, 435–439.

Sugita, T., Shiraki, K., Ueda, S., Iwa, N., Shoji, M., 1984. Induction of acute myoclonic encephalopathy in hamsters by subacute sclerosing panencephalitis virus. J. Infect. Dis. 150, 340–347.

Wheeler, J.G., Wofford, J., Turner, R.B., 1993. Historical cohort evaluation of ribavirin efficacy in respiratory syncytial virus infection. Pediatr. Infect. Dis. J. 12, 209–213.

Yalaz, K., Anlar, B., Oktem, F., Aysun, S., Ustacelebi, S., Gurcay, O., Gucuyener, K., Renda, Y., 1992. Intraventricular interferon and oral inosiplex in the treatment of subacute sclerosing panencephalitis. Neurology 42, 488–491.

Yoshioka, H., Nishimura, O., Nakagawa, M., Ochi, M., Takeuchi, Y., Tominaga, M., Hasegawa, K., Osamura, T., Goma, H., Sawada, T., Imanishi, J., 1989. Administration of human leukocyte interferon to patients with subacute sclerosing panencephalitis. Brain Develop. 11, 302–307.