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Short communication

# Effect of recombinant human interferon α B/D (rHu-IFN-α B/D) in combination with acyclovir in experimental HSV-1 encephalitis

U. Wintergerst [a,*], J.D. Gangemi [b], R.J. Whitley [c], S. Chatterjee [d], E.R. Kern [c]

[a] *Department of Pediatrics, University of Munich, Munich, Germany*
[b] *Biomedical Alliance, University of South Carolina, Clemson, SC, USA*
[c] *Department of Pediatrics, University of Alabama, Birmingham, AL, USA*
[d] *JCR Biopharmaceuticals Inc., San Diego, CA, USA*

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**Abstract**

The efficacy of recombinant human interferon α B/D in experimental HSV-1 encephalitis was investigated in the murine system. Recombinant Hu-IFN-α B/D significantly reduced the mortality in a mouse encephalitis model (about 30%, $P = 0.021$), whereas natural mouse interferon was inactive. Combination of acyclovir with Hu-IFN-α B/D had an additive effect. 

*Keywords:* Encephalitis; HSV-1; Interferon; Acyclovir

HSV causes a broad spectrum of disorders ranging from asymptomatic infection to life threatening diseases (e.g. neonatal sepsis, encephalitis) (Whitley, 1988). Antiviral drugs like acyclovir (ACV) have demonstrated efficacy in HSV encephalitis, but are still not optimal (Sköldenberg et al., 1984; Whitley et al., 1986). The remaining mortality in ACV treated patients is about 20–30%, and 50% of the survivors are neurogically damaged.

Interferons are potent antiviral substances (Chatterjee et al., 1985; Chatterjee and Burns, 1990) which may offer therapeutic benefits, particularly in combination with nucleoside analogues (Stanwick et al., 1981; Moran et al., 1985). Beneficial effects of interferon therapy in HSV infections were observed in an HSV-2 sepsis model with mouse α or β interferon in combination with DHPG (Fraser-Smith et al., 1984a,b), in an HSV-1 skin infection model with interferon α A/D and acyclovir (Cerruti et al., 1985) and in an HSV-1

* Corresponding author. Present address: Children's Hospital of the Ludwig-Maximilian's University, Lindwurmstr. 4, 80337, Munich, Germany. Tel.: +49-89-51603931; fax: +49-89-51603964.

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pneumonia model with Hu-IFN-α B/D (Gangemi et al., 1989). However, the in vivo efficacy in HSV-1 encephalitis is not well-established. Reports with interferon inducers in HSV-1 encephalitis are contradictory (Catalano et al., 1972; Fitzwilliam and Griffith, 1976). In a study of De Clercq and Luczak (1976) mouse interferon did not alter the course of disease. Recently, we and others demonstrated the cross-species activity of the rHu-IFN-α B/D against HSV-1 replication in mouse fibroblast cells (Horisberger and De Staritzky, 1987; Chatterjee and Burns, 1990; Wintergerst et al., 1996). In an extension of a retrospective study which indicated some beneficial effect of human B interferon in HSV encephalitis in humans (Wintergerst and Belohradsky, 1992) we investigated the effect of rHu-IFN-α B/D with and without acyclovir on HSV-1 replication in a mouse encephalitis model. Additionally, natural mouse interferon was used as a control.

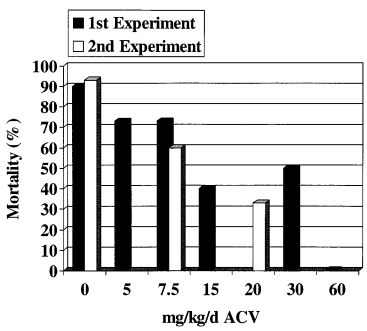


Fig. 1. Effect of different doses of acyclovir on the mortality of mice infected intranasally with HSV-I (results of two independent experiments).

Table 1
Effect of interferons and combination therapy of interferon with acyclovir on the mortality of mice infected intranasally with HSV-1 ($n = 30$)

| Treatment | Mortality | $P$-value | MDD[c] |
|---|---|---|---|
| Placebo | 28/30 (93%) | – | 6.4 |
| Mouse interferon[a] | 13/15 (87%) | N.s.[d] | 6.2 |
| Hu-IFN-α B/D[a] | 20/30 (67%) | 0.021[e] | 7.1 |
| ACV[b] | 22/30 (73%) | N.s. (0.08[e]) | 8.2 |
| Hu-IFN-α B/D+ACV | 14/30 (47%) | N.s. (0.06[f], 0.19[g]) | 8.0 |

[a] $2 \times 100\,000$ IU/day i.p. for 5 days.
[b] $1 \times 7.5$ mg/kg/day for 5 days.
[c] MDD: mean day of death (n.s. vs. placebo).
[d] N.s.: not significant.
[e] Compared with placebo.
[f] Compared with ACV.
[g] Compared with Hu-IFN-α B/D.

To determine the in vivo efficacy of rHu-IFN-α B/D, 3-week-old swiss webster mice were infected intranasally with the neurotropic HSV-1 strain E-377 (Moran et al., 1985) as a model for herpes simplex encephalitis (De Clercq and Luczak, 1976). Each animal was inoculated with approximately $2 \times 10^5$ pfu and the volume of the inoculum was 40 μl. The $LD_{50}$ for that virus was $1.5 \times 10^4$ pfu.

Treatment was initiated either 24 or 48 h after infection. Interferons (rHu-IFN-α B/D or mouse interferon) and acyclovir (ACV) were administered intraperitoneally for 5 days. The doses for both interferons were $2 \times 3300$, $2 \times 10\,000$, $2 \times 33\,000$, and 100 000 U/day. ACV was administered with increasing doses beginning at 5–60 mg/kg/day once daily.

Mortality was checked daily for 21 days. For statistical analysis, Fisher's exact test and two-sided $P$-values were used. In addition, combination therapy of rHu-IFN-α B/D, mouse interferon and acyclovir was also investigated.

Recombinant Hu-IFN-α B/D, but not mouse interferon, reduced significantly the mortality in mice when treatment was initiated 24 h after infection at a dose of $2 \times 100\,000$ U/day (Table 1, $P = 0.021$). In addition, the mean day of death was somewhat increased in the rHu-IFN-α B/D group vs. placebo but did not reach significance. Doses of rHu-IFN-a B/D less than $2 \times 100\,000$ IU/day and treatment started at 48 h after infection were not effective (data not shown). This effect is remarkable because the human interferon α B/D was more effective than the natural mouse interferon. In a previous study from De Clercq's laboratory natural mouse interferon (105–106 IU/mouse) also failed to protect HSV-1-infected mice when given 24 h after intranasal infection (De Clercq and Luczak, 1976). In this study similar doses of interferon (approximately $10 \times 106$ U/kg)

were used. It is well-known that most interferons cross the blood–brain barrier only to a small extent. One reason for the better activity of rHu-IFN-α B/D might be that it penetrates somewhat better through the blood–brain barrier than mouse interferon. However, at present we have no experimental data to support this hypothesis.

ACV monotherapy reduced the mortality in a dose-dependent manner (Fig. 1). The mortality was reduced by 50% at a dose of 15–30 mg/kg/day, i.e. quite similarly to a study of Smee et al. (1985) where 40% of mice intracerebrally infected with HSV-1 survived following ACV monotherapy at a dose of 40 mg/kg/day. The slight differences between this study and our results might be explained by the different routes used for HSV-1 infection and drug administration. Smee et al. (1985) administered acyclovir subcutaneously and not intraperitoneally.

In combination with a ACV dose of 7.5 mg/kg/day, which led reproducibly to a mortality rate of 60–70%, rHu-α B/D appeared to have an additive effect (Table 1). Exact calculations for synergy, however, could not be performed because no dose–effect relationship could be established for rHu-α B/D-IFN.

Our findings suggest that recombinant human interferon (rHu-IFN-α B/D) may modify the course of HSV-1 encephalitis in humans.

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