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Short communication

Protective effects of murine recombinant interferon-β administered by intravenous, intramuscular or subcutaneous route on mouse hepatitis virus infection

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Abstract

The significance of the route for administration of murine recombinant interferon-β (IFN-β) for inducing its therapeutic effects has been studied. BALB/c mice were daily injected intravenously, intramuscularly or subcutaneously with 1.5×10^3, 1.5×10^4, or 1.5×10^5 IU of IFN-β, from one day before to 8th day after mouse hepatitis virus (MHV-2) challenge. All mice received IFN-β survived significantly longer than those without IFN. In the liver of those IFN-treated mice, viral growth and the histopathological damages were extremely alleviated. These results suggest that, irrespective of the differences in the route of administration, IFN-β markedly suppressed viral activity when its administration was started prior to viral infection. For clinical use, however, further studies are needed on the optimal route for administration if IFN-β is given after viral infection.

Keywords: Murine IFN-β; Mouse hepatitis virus; IFN administration route

Of three types of interferon (IFN), α, β and γ, the former two IFNs are intrinsically induced during an early phase of the viral infection and play an important role in host defense mechanisms. The antiviral effects of IFNs have been demonstrated following administration of exogenous IFNs in a variety of virus infections (Cirelli and Tyring, 1995). IFN-induced antiviral pathways are mediated by 2′,5′-oligoadenylate (2-5A) synthetase, double-stranded RNA-dependent

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protein kinase (RPK) and Mx protein (Stark et al., 1998). The 2-5A synthetase induces the cleavage of single-stranded viral RNA by activating 2-5A-dependent RNase L (Kerr and Brown, 1978). The RPK shows inhibitory effects on viral protein synthesis by inactivating eLF-2α function (Meurs et al., 1990). The Mx protein interferes with the replication of influenza and other negative-stranded RNA viruses at the virus transcription and at other steps (Arnheiter et al., 1996). In addition, some other antiviral effector proteins are also synthesized in response to IFN (Stark et al., 1998). In animals IFNs display antiviral effects through their immunomodulatory activity, such as activation of macrophages and natural killer cells (Cirelli and Tyring, 1995).

Mouse hepatitis virus (MHV), a member of the *Coronaviridae*, induces different kinds of diseases such as hepatitis, encephalomyelitis and enteritis, depending on the strain (Compton et al., 1993). All types of IFN have been reported to play important roles in protecting mice from MHV infections. IFN-γ is protective in hepatitis and encephalitis caused by different MHV strains (Smith et al., 1991; Zhang et al., 1997; Kyuwa et al., 1998; Parra et al., 1999). IFN-α and -β also exhibit similar protective effects (Virelizier and Gresser, 1978; Minagawa et al., 1987; Smith et al., 1987). Taken together, these results suggest that the MHV-mouse system is a highly sensitive animal model useful for the antiviral study of IFNs.

IFNs have been used for therapeutic and prophylactic purpose in a number of human viral infections and various cancers (Cirelli and Tyring, 1995). The antiviral effects of IFNs appeared to be influenced by the IFN type, and the dose and route of administration. Several investigators have insisted that the selection of the IFN administration route is important for IFN to induce sufficient therapeutic efficacy and to alleviate its side effects, (Wills et al., 1984; Rutenfranz and Kirchner, 1988; Alam et al., 1997), while others failed to find such differences in the effects of different administration routes (Merritt et al., 1986; Chiang et al., 1993; Salmon et al., 1996). These controversial results appeared to be due to the differences in experimental conditions, such as the sensitivity of the evaluation methods and animals used. In the present study, one has attempted to evaluate the anti-MHV effect of the murine recombinant IFN-β administered by three different routes: intravenous (i.v.), intramuscular (i.m.) or subcutaneous (s.c.) route. The i.v. administration rapidly distributed IFN to the liver lesions where the MHV infection takes place, while the i.m. and s.c. routes distributed IFN more slowly.

Five-week-old male BALB/c mice purchased from the Charles River Japan (CRJ, Hamamatu, Japan), with body weights ranging from 21 to 25 g, were used in this study. These mice were serologically free from MHV and other murine pathogens. The murine recombinant IFN-β used in this study was a product (3×10^6 IU/ml) of the Pharmaceutical Research Laboratory, Toray Industries, (Matsuda et al., 1986, Toray, Kamakura, Japan). The mice were treated daily i.v., i.m., or s.c. with a dose of 50 μl IFN-β, 1.5×10^3, 1.5×10^4 or 1.5×10^5 IU, from 1 day before to 8 days after MHV challenge. These doses of IFN did not show any side effects on mice, although 10 times higher concentrations (1.5×10^6 IU) induced mild side effects, such as fever, leukocytopenia and plateletpenia. In the present study, we have not started IFN treatment after the MHV infection, since the post infection treatment has been reported to be less effective in MHV-2 infection (Minagawa et al., 1987). These mice were infected i.p. on day 0 with 5×10^3 PFU of MHV-2, a highly virulent strain with hepatotropism and considerably resistant to IFN compared with other MHV strains (Taguchi et al., 1976; Taguchi and Siddell, 1985). This dose of MHV-2 corresponds to more than 10^3 LD50 for 5-week-old BALB/c mice. To statistically analyze the data, the Kaplan–Meier method was used for calculation of survival time and the log-rank test for the differences in mortality. As shown in Table 1, mice treated with IFN of 1.5×10^4 and 1.5×10^5 IU survived significantly longer than those untreated, irrespective of the difference in IFN administration route. Some mice that received IFN (1.5×10^5 IU) survived during the observation period (8 days), while all untreated mice died within 2 days after MHV challenge (Fig. 1). The log-rank test indicated a significant difference in survival time between the IFN-treated and un-

Table 1
Survival rate and time of mouse hepatitis virus (MHV-2) infected-mice treated with interferon (IFN-β)

Administration route	Dose of IFN-β (IU/mouse)			
	0	1500	15 000	150 000
i.v.	0/6[a]	0/6	0/6	2/6
	2.0 ± 0.0[b]	3.3 ± 0.2***,[d]	4.7 ± 0.2***, [e]	7.0 ± 0.4***, [f]
s.c.	ND[c]	0/6	0/6	2/6
		2.3 ± 0.2NS[d]	3.8 ± 0.3***,[c]	6.3 ± 0.6***, [f]
i.m.	ND	0/6	1/6	4/6
		3.0 ± 0.4*, [d]	5.5 ± 0.6***, [e]	7.4 ± 0.4***, [f]

[a] Number of surviving/number of total mice as examined on day 8 after MHV-2 infection.
[b] Mean survival time ± S.D. (in days).
[c] ND, not done.
[d, e, f] Not significantly different in each group ($P > 0.05$).
*** $P < 0.001$; * $P < 0.005$, significantly different by log-rank test compared to the value for untreated mice.

treated mice, but no significant difference among mice given IFN by different routes (Table 1). Similarly, there were no significant differences in mortality among mice administered IFN by i.v., i.m. and s.c. routes when judged on days 5–8 postinfection, while there was a significant difference between the mice treated with IFN and those that were left untreated (Fig. 1). These results have indicated that the IFN-β is protective against MHV-2-induced hepatitis and the protective effect is not significantly influenced by the difference in administration route.

Next, it has been examined whether the protection truly results from the suppression of virus growth in the liver, a major target of MHV-2 (Taguchi et al., 1976). Mice treated with 1.5×10^5 IU IFN and untreated mice were killed on days 2 and 5 after MHV infection. The virus titers were measured by plaque assay (Taguchi et al., 1976). As shown in Fig. 2, a striking difference was found in the virus titer in the liver between IFN-treated and untreated mice. When assayed on day 2, the virus titer of untreated mice was more than 10^6 PFU/0.1 g liver, while in treated mice the virus titer was under the detection level (< 0.5 in log 10) in six of nine mice and less than 10^2 PFU/0.1 g in the remaining three. Tukey–Kraner statistical analysis demonstrated a significant difference between virus titers in untreated mice and those treated with IFN by either route ($P < 0.005$). However, there was no difference in virus titers among mice treated with IFN by either of the three different routes ($P > 0.05$). When assayed on day 5, the virus titers of the IFN-treated mice showed substantial differences: more than half of mice had 10^4–10^5 PFU, while other mice

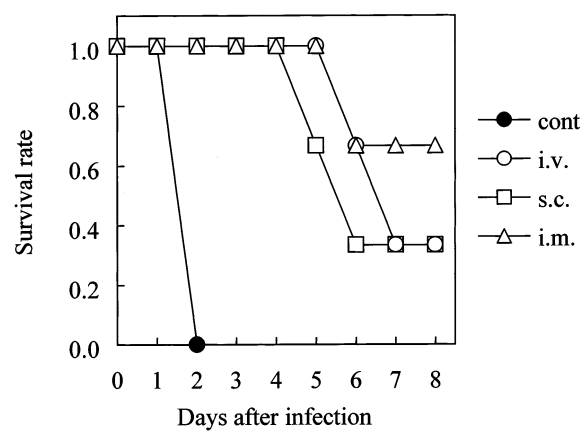

Fig. 1. Survival curves for the interferon (IFN)-treated mouse hepatitis virus (MHV-2)-infected mice. Mice were daily treated with i.v. (○), i.m. (△) or s.c. (□) 1.5×10^5 IU IFN-β from 1 day before to 8 days after the infection with 5×10^3 PFU of MHV-2. Control mice (cont, ●) were daily injected by the i.v. route with 50 μl of PBS. Mortality was daily checked by day 8 postinfection. Long-rank analysis showed a significant difference in mortality on days 5–8 between IFN-treated mice (○, △, □) and untreated mice (●) ($P < 0.05$), while there were no significant differences among mice treated by different routes (○, △, □) ($P > 0.05$).

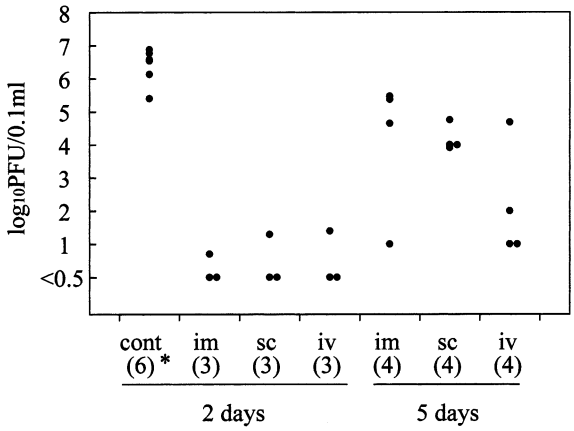

Fig. 2. Mouse hepatitis virus (MHV-2) titers in the liver of interferon (IFN)-administered mice. Mice daily treated i.v., i.m. or s.c. with 1.5×10^5 IU of IFN from 1 day before to 5 days after MHV challenge were killed on days 2 and 5. Untreated mice were killed on day 2. The virus titers in the liver of individual mice were measured by plaque assay. The number in parenthesis indicates the number of mice sacrificed. Tukey–Kraner statistical analysis showed a significant difference in titers on day 2 between IFN-treated and untreated mice ($P < 0.005$); however, no significant differences were observed among mice treated by different routes both on days 2 and 5 ($P > 0.05$).

had less than 10^3 PFU. These differences could be accounted for by differences in the individual mouse response to MHV-2. In spite of the variation, statistical analysis indicated no significant difference in viral titers among mice given IFN by different routes ($P > 0.05$).

Histopathological examination of the liver on day 2 post infection demonstrated that the IFN-untreated mice suffered from fulminant hepatitis, containing numerous necrotic hepatocytes and severe hemorrhage, while in IFN-treated mice only a small number of tiny lesions consisting of inflammatory cells were detected (Fig. 3). When examined on day 5, the liver lesion area was found to be enlarged in these mice (Fig. 3). However, in several mice the liver lesions remained as thin as those found on day 2. The histopathological variation appeared to reflect to the variation in the virus titer shown in Fig. 2.

It has been reported that the optimal pharmacokinetic response of human IFN-β is attained in volunteers by i.m. rather than s.c. or i.v. administration (Alam et al., 1997). In the present study, however, the murine IFN-β effectively prevented the virus replication in the liver without showing apparent difference in the administration route, suggesting that the pharmacokinetic response of murine IFN-β is independent of the administration route. The findings are in agreement with those of Salmon et al. (1996), who described that in volunteers human IFN-β has a similar extent and duration of clinical and biological effects independent of the route of administration. Chiang et al. also described that the antiviral activity of human IFN-β administered i.v., i.m. and s.c. was not different among these groups as measured by reduction in viremia and skin rash after infection of monkeys with simian varicella virus (1993). These observations suggest that the IFN-β is antivirally effective independent of the route of administration.

In the present study, it has been found that the IFN-β almost thoroughly prevented viral replication in the liver during the first 2 days after the infection, but allowed the replication later than day 5. This is due probably to the insufficiency of immune responses against viral components, such as neutralizing antibodies and cytotoxic T cells that prevent the viral growth in combination with the direct action of IFNs. Alternatively, mutated viruses resistant to IFN might emerge in the later stage of infection. These mutated viruses might possibly cause the lethal hepatitis seen in the IFN-treated mice. This possibility is currently under investigation.

Minagawa et al. (1987) reported that most of mice administered with the IFN-β of 8×10^3 IU or higher survived the lethal hepatitis caused by MHV-2. In contrast, it was found that the IFN-β protected only 33–66% of mice from death even when as much as 1.5×10^5 IU were used. The differences between the findings and theirs are due probably to: (1) the mouse strain used in each experiment; (2) the dose of MHV-2 used for the challenge; and (3) the route of IFN administration. They used 5-week-old C57BL/6 mice and inoculated 100 PFU of MHV-2 for challenge. It

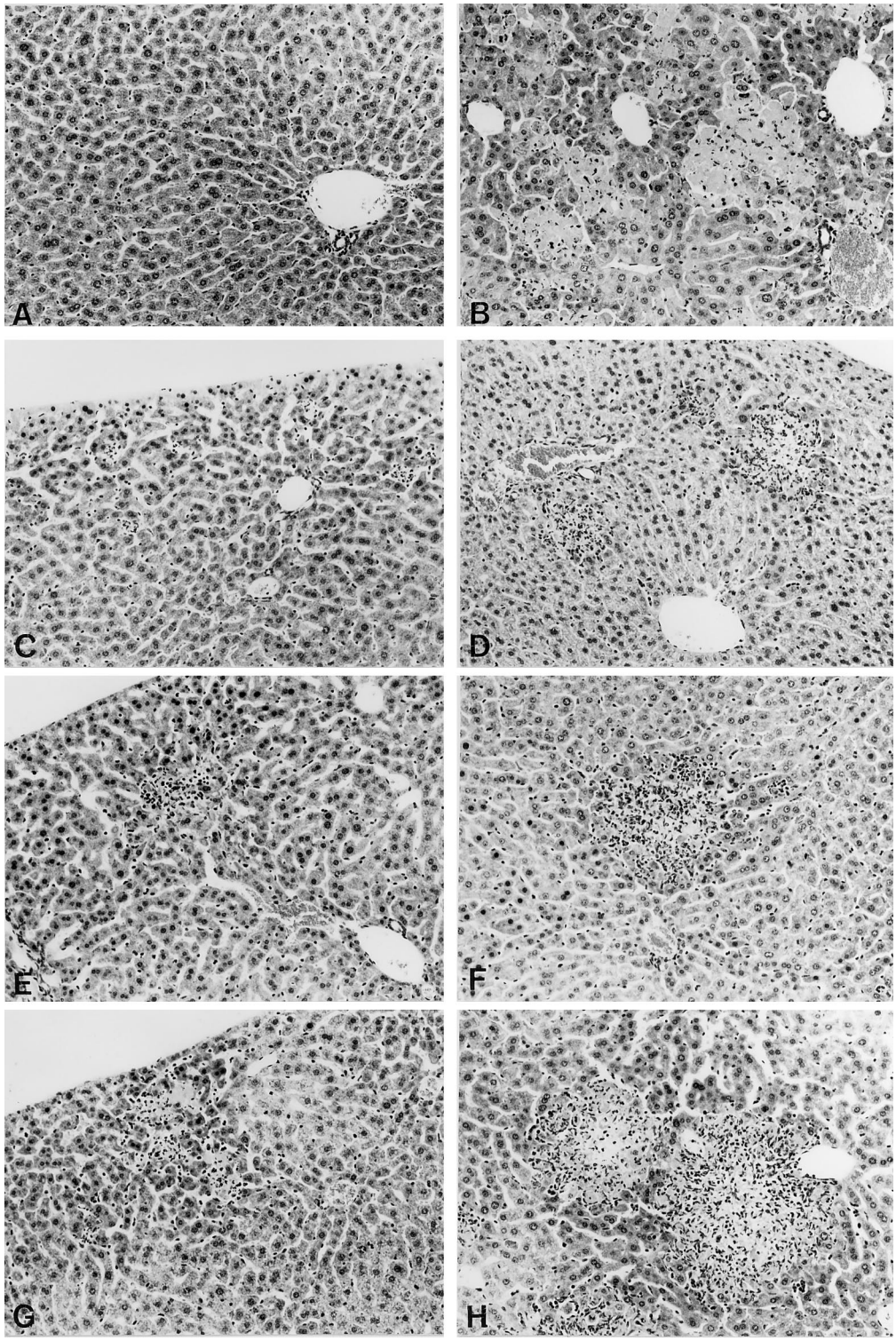

Fig. 3. Histopathological changes in the liver of mice given interferon (IFN) by different routes. Liver specimens from mice shown in Fig. 2 were histopathologically examined following HE staining. Mice were treated i.v. (C, D), i.m. (E, F) or s.c. (G, H) with 1.5×10^5 IU of IFN and sacrificed on days 2 (C, E, G) and 5 (D, F, H) after mouse hepatitis virus (MHV-2) infection. Untreated mice (B) were examined on day 2. Uninfected mouse liver is shown as a negative control (A).

has been previously described that the mice inoculated with virus titers ranging $10–10^5$ PFU of MHV-2 showed mortality to the same extent (Taguchi et al., 1976), suggesting that the virus titers are not responsible for the difference. Minagawa et al. administered IFN by the i.p. route. The i.p. and i.v. routes of administration, however, seem to be very similar in terms of the mode of IFN distribution; both routes supply it to the liver and spleen very rapidly. Thus, the difference in mouse strains used in each experiment is most likely responsible for the different outcome. The C57BL/6 strain used by Minagawa et al is a high responder to MHV, producing a high titer of antiviral antibodies (Wada et al., 1981; Nakanaga et al., 1983). The combination of IFN and neutralizing antibodies could successfully protect C57BL mice from a lethal MHV-2 infection.

In the MHV-2-infected mice, a relatively high level of IFN was detected in the blood in an early phase of infection (Taguchi et al., 1976). However, the significance of this IFN for protection is not obvious, although IFN and other cytokines produced in an early stage of low-virulent MHV infection is known to confer resistance to mice against highly virulent MHV-2 by activating macrophages (Taguchi et al., 1980). The antiviral effects of IFN-β shown in this study could be mediated, in part, by the activated macrophages or natural killer cells in addition to the direct antiviral effect of IFN-β on the virus replication in hepatocytes.

IFNs are clinically used for the treatment of acute hepatitis caused by hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) (Cirelli and Tyring, 1995). IFN-α is applied for all of these diseases, but its protective effect depends on the causative hepatitis virus (Cirelli and Tyring, 1995), while IFN-β is predominantly used for acute HCV infection with remarkable protective effects after i.v. administration (Omata et al., 1991). The present findings of the effect of IFN-β on acute mouse hepatitis suggest that the administration route is not important for anti-HCV efficacy, at least if IFN treatment is started prior to the virus infection.

In conclusion, the present study demonstrated that when IFN-β was administered i.v., i.m., or s.c. into BALB/c mice from 1 day before MHV-2 infection, the virus titers and histopathological changes in the liver were significantly reduced, irrespective of the differences in the administration route. Thus, for induction of antiviral efficacy, the administration route of IFN appears not to be important. In individuals who are already infected, such as patients infected with HBV or HCV, the timing rather than the route of administration may be one of the important factors that affects the clinical efficacy of IFN. To this end work is now in progress whereby IF is administered after the viral infection to establish the most effective administration.

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