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Antiviral activity of recombinant interferon-α on hepatitis A virus replication in human liver cells

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Abstract

Human recombinant interferon-α (IFN-α) was assayed for its antiviral effect on hepatitis A virus (HAV) replication in the human hepatoma cell line PLC/PRF/5. IFN-α resulted in concentration-dependent reduction of HAV antigen expression and HAV replication. IFN-α had a prophylactic effect, but was still effective when it was added after the infection, even at the end of the first replication cycle. An important increase in 2′,5′-oligoadenylate synthetase activity in the IFN-treated human liver cells was observed. The antiviral effect of IFN-α could be attributed to the induction of this enzyme. Moreover we have shown that IFN-α and glycyrrhizin were synergistic in their antiviral actions against HAV. IFN-α emerged, from the present study, as a promising candidate for chemotherapy of severe forms of hepatitis A.

Keywords: Hepatitis A virus; Anti-HAV substance; 2′,5′-Oligoadenylate synthetase; Interferon treatment

1. Introduction

Hepatitis A virus (HAV) is a hepatotropic picornavirus classified within the genus Hepatovirus (Francki et al., 1991). Infection with HAV remains a worldwide health

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problem. In the United States, at least 25,000 cases of hepatitis A are reported annually (Gust, 1988). In many developing nations, improved sanitation has increased the population of susceptible adults. In this population severity of illness increases with age and the case-fatality rate is 2.7% over age 49 years (Hadler et al., 1991). Relapses in liver inflammation occur more frequently and in some cases progression to hepatic fulminant failure requires liver transplantation.

Killed vaccine is now available (André et al., 1992). However it is expensive and large-scale vaccination is not yet possible. Recent research efforts have focused on the development of effective antiviral compounds (Widell et al., 1986; Biziagos et al., 1987, 1990; Superti et al., 1987, 1989; Passagot et al., 1988; Crance et al., 1990, 1994b; Girond et al., 1991; Divizia et al., 1992).

Among the antiviral substances screened in our laboratory (Biziagos et al., 1987; Passagot et al., 1988; Crance et al., 1990, 1994b; Girond et al., 1991), recombinant interferon-α (IFN-α) has proved an attractive candidate as anti-HAV drug. This compound exhibited potent antiviral activity against HAV replication at concentrations which were at least 1000-fold lower than the cytotoxicity concentration (Crance et al., 1994b). IFN-α is now produced by pharmaceutical laboratories and is available for antiviral treatments. Its antiviral activity was shown on several viruses (Finter et al., 1991). Moreover IFN-α has already been used for its therapeutic effects on chronic hepatitis B and C (Hoofnagle et al., 1986; Perillo et al., 1990).

The aim of the present work was to study the antiviral activity of IFN-α on HAV replication in human liver cells under different experimental conditions and determine its mechanism of action in these cells.

2. Materials and methods

2.1. Antiviral compound

IFN-α_{2a} was purchased from Roche Laboratories (Paris, France). It was dissolved in RPMI 1640 medium before use.

2.2. Cell culture

Human hepatoma cell line PLC/PRF/5 (obtained from the American Type Culture Collection, Rockville, MD) was grown at 37°C in 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) and containing 100 IU/ml of penicillin and 100 μg/ml of streptomycin. HAV-infected cells were maintained, at 32°C, in RPMI 1640 medium without FCS.

2.3. Virus

The CF53 strain of HAV was isolated in our laboratory (Crance et al., 1985a) and adapted to PLC/PRF/5 cells by serial passage (Crance et al., 1985b). The virus was passaged 28 times in these cells before use. After 12 days of incubation at 32°C,

virus-infected cells were frozen and thawed 4 times and the virus suspension was clarified by centrifugation at 10,000 *g* for 20 min at 4°C. This virus pool contained $10^{7.0}$ 50% tissue culture infective doses ($TCID_{50}$s) per ml.

2.4. Antiviral assays and cytotoxicity evaluation

For determination of antiviral activity, confluent monolayers in 24-well tissue culture plates were infected at the appropriate multiplicity of infection (m.o.i.). After 1 h adsorption, inoculum was eliminated and medium was added in the presence of various concentrations of IFN-α (4 wells/concentration). For each panel, quadruplicate wells were used as virus controls and cell controls. The infected cells were incubated at 32°C for 12 days (multiple rounds of replication). The virus was then extracted by 4 cycles of freezing and thawing and antiviral activity was determined by the inhibition of viral antigen expression and by the reduction of virus yield. Viral antigen expression was evaluated by solid-phase radioimmunoassay (RIA) endpoint titration (Crance et al., 1987). The drug concentration required to reduce viral antigen expression by 50% in comparison to the control was designated as 50% effective dose (ED_{50}). The infectious virus titer was determined by titration in 48-well tissue culture plates (Crance et al., 1990).

For determination of cytotoxicity, confluent monolayers in 24-well tissue plates were exposed to different concentrations of IFN-α (4 wells/concentration) in maintenance medium for 12 days at 32°C. Each medium containing IFN-α at the appropriate concentration was renewed every 2 days. Four wells were used as controls (non-drug-treated cells). After 12 days of incubation, cytotoxicity was evaluated by determining the inhibition of Trypan blue exclusion, as described previously (Crance et al., 1990). The 50% cytotoxic dose (CD_{50}) was defined as the concentration required to reduce cell viability by 50%.

2.5. Effect of time of addition of IFN-α on virus yield from single-round replication

IFN-α at 10,000 IU/ml was added to monolayers of PLC/PRF/5 cells in 24-well tissue culture plates, at various times before or after addition of virus (m.o.i. = 1.0) and the virus yield was determined after a 96 h single cycle of virus replication (Crance et al., 1994a).

Three types of experiments were performed. First, cells were exposed to IFN-α in maintenance medium for 24 h at 32°C. The drug solution was discarded and the cells were washed 3 times, then inoculated with HAV (pretreatment). The monolayers were then incubated with IFN-α-free maintenance medium at 32°C for 96 h. Second, monolayers were pretreated as described above. Then the cells were inoculated with HAV in presence of IFN-α and incubated in culture medium containing IFN-α for 96 h (pre- and post-treatment). Third, PLC/PRF/5 cells were inoculated with HAV, and immediately after adsorption or at various times postinfection (0, 6, 12, 18, 24, 36, 48, 60 and 96 h) IFN-α was added and monolayers were incubated with the drug at 32°C until 96 h postinfection (post-treatment). All tests were run in triplicate. At 96 h postinfection, cells were washed and HAV was extracted by freezing and thawing and the HAV infectious titer was determined in cell culture.

2.6. Antiviral activity of IFN-α in HAV-infected PLC / PRF / 5 cells after a single cycle of virus replication

PLC/PRF/5 cells were inoculated with HAV (m.o.i. = 1.0) and incubated at 32°C until 96 h postinfection during a single cycle of virus replication (Crance et al., 1994a). Then, IFN-α was added and monolayers were incubated at 32°C for different times (multiple rounds of replication). The virus was then extracted by 4 cycles of freezing and thawing and antiviral activity was determined by the reduction of infectious virus titer.

2.7. 2',5'-Oligoadenylate synthetase activity assay

IFN-α was also examined for its effect on 2',5'-oligoadenylate synthetase (2',5'-AS) activity in PLC/PRF/5 cells (single-cycle conditions). Confluent cells in 75 cm^2 flasks were washed and infected by HAV at an m.o.i. of 1.0. After 60 min of adsorption at 4°C, all flasks were washed twice with maintenance medium. The cells were then treated by different concentrations of IFN-α (2 flasks/concentration) and incubated at 32°C for 96 h; two flasks were used as controls (virus-infected non-drug-treated cells). On the other hand, non-infected 75-cm^2 flasks were treated by different concentrations of IFN-α (2 flasks/concentration) and incubated at 32°C for 96 h; two flasks were used as controls (virus-non-infected non-drug-treated cells).

2',5'-AS was assayed on PLC/PRF/5 cells in 75 cm^2 flasks as previously described (Chousterman et al., 1983). Briefly, cells were washed twice with buffer A: 140 mM NaCl, 3 mM $MgCl_2$, 35 mM HEPES, pH 7.5. Then, cells were lysed for 10 min at 4°C in buffer B: 20 mM Tris, pH 8.0, 25 mM KCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.5% NP40 (10^7 cells/ml). After centrifugation, cell supernatants were incubated for 15 min at room temperature with 0.05 ml of poly(rI) · (rC) sepharose beads (prepared in our laboratory). The beads were centrifuged and washed 3 times with buffer B and incubated for 2 h at 37°C in 10 μl of the same buffer containing 0.1 mg bovine serum albumin, 0.1 mg/ml creatine kinase, 5 μg/ml poly(rI) · (rC), 5 mM $Mg(OAc)_2$, 6 mM creatine phosphate, 3 mM ATP and 0.05 μCi [α-^{32}P]ATP. After a 2-h incubation at 37°C, the reaction was stopped by the addition of 20 mM EDTA. The beads were centrifuged and 5 μl of the supernatants were spotted on a PEI-cellulose thin layer plate. The chromatogram was developed in 2 M Tris HCl, pH 8.6. The spots containing ATP and the 2',5'-oligoadenylates formed were localized by exposure to X-ray film, cut and counted. The specific activity of the 2',5'-AS is defined as units per 10^7 cells (one enzyme unit = 1 nmol AMP residues incorporated into 2',5'-oligoadenylates per min at 37°C).

In a parallel experiment, HAV infectious titer from infected PLC/PRF/5 cells (in the conditions described above) in other 75 cm^2 flasks was determined in cell culture.

2.8. Antiviral activity of IFN-α in combination with glycyrrhizin

Confluent monolayers of PLC/PRF/5 cells in 24-well plates were inoculated in quadruplicate with HAV at an m.o.i. of 1.0 $TCID_{50}$/cell. After a 60-min adsorption

period at 4°C, inoculum was eliminated and RPMI containing IFN-α, glycyrrhizin or combinations of these two drugs were added to the wells. After incubation at 32°C for 12 days, each medium being renewed every 2 days, HAV from quadruplicate wells was extracted by 4 cycles of freezing and thawing and antiviral activity was determined by the reduction of virus yield. The effectiveness of the drug combination against HAV was evaluated by calculating: (1) the enhancement of the antiviral activity compared with that of IFN-α alone; and (2) the synergistic interaction, which was defined as a decrease in infectious virus yield that was greater than the algebraic sum of the decreases observed with single drugs (Hayden et al., 1980; Biziagos et al., 1990).

2.9. Statistical analysis

Statistical analysis of the data was carried out using Student's *t*-test and one-way analysis of variance (ANOVA).

3. Results

3.1. Inhibitory effect of IFN-α on HAV antigen expression

Inhibition of the HAV antigen expression, measured 12 days after infection, at 3 m.o.i. (1.0, 0.1 and 0.01 $TCID_{50}$/cell) in the presence of various non-toxic concentrations of IFN-α, is shown in Table 1. For each m.o.i. tested, a dose-dependent inhibition of expression of HAV antigen in PLC/PRF/5 cells was observed (one-way ANOVA, $P < 0.05$). The inhibitory effect of IFN-α was also dependent on the m.o.i. As the m.o.i. was decreased, the antiviral effect of the drug increased. The concentrations required to inhibit HAV antigen expression by 50% (ED_{50}) were 90, 34 and < 10 IU/ml at an m.o.i. of 1.0, 0.1 and 0.01, respectively.

Cytotoxicity was evaluated by Trypan blue staining (Table 2). All of the used concentrations were non-toxic (*t*-test; $P > 0.05$). The CD_{50} value of IFN-α for cellular

Table 1
Inhibitory effect of IFN-α on the expression of HAV antigen in PLC/PRF/5 cells at three multiplicities of infection [a]

m.o.i.	HAV antigen titer (% of control) [b]				
	IFN-α concentration (IU/ml)				
	0	10	100	1000	10,000
1.0	100.0 ± 16.3	83.1 ± 15.0	45.7 ± 17.1	11.4 ± 1.2	0.7 ± 0.4
0.1	100.0 ± 24.7	59.9 ± 7.0	23.5 ± 2.2	2.5 ± 0.6	0
0.01	100.0 ± 10.6	32.9 ± 7.2	8.8 ± 2.5	0	0

[a] PLC/PRF/5 cells were infected with HAV at the appropriate m.o.i. in the presence of IFN-α and further incubated at 32°C for 12 days. On day 12, antiviral activity was evaluated by the determination of the HAV antigen titer by RIA endpoint titration.
[b] Means ± S.D. of quadruplicate experiments.

Table 2
Cellular viability after IFN-α treatment [a]

IFN-α concentration (IU/ml)	Cell viability [b] (% of control)
0	100.0 ± 5.6
10	96.5 ± 6.4
100	94.6 ± 7.9
1000	93.7 ± 8.0
10,000	109.5 ± 7.7

[a] PLC/PRF/5 cells were treated with different concentrations of IFN-α and further incubated at 32°C for 12 days. On day 12, toxicity was evaluated by Trypan blue staining.
[b] Means ± S.D. of quadruplicate experiments.

viability was $> 10^4$ IU/ml (Table 2). The selectivity indices, calculated as CD_{50}/ED_{50} ratios were > 100 > 200 and > 1000 at an m.o.i. of 1.0, 0.1 and 0.01, respectively. These results indicate that IFN-α proved to be highly selective in its antiviral action. On the other hand, the effect of IFN-α on cell growth at 37°C was determined over 3 days. At 1000 IU/ml of IFN-α, the cell growth was not significantly reduced (not shown in Table 2). The concentration of IFN-α resulting in 50% reduction of cell replication on day 3 was 8800 IU/ml.

3.2. Inhibitory effect of IFN-α on HAV replication

IFN-α was also examined for its inhibitory effect on HAV infectivity at 3 different m.o.i. (1.0, 0.1, 0.01 $TCID_{50}$/cell). The infected cells were incubated in the presence of various concentrations of IFN-α for 12 days (multiple rounds of replication). The results are shown in Table 3. For the 3 m.o.i., IFN-α caused a concentration-dependent reduction in HAV infectivity (one-way ANOVA, $P < 0.05$). The drug significantly reduced the infectious titer at a concentration as low as 10 IU/ml at an m.o.i. of 0.01. At 10,000 IU/ml of IFN-α (the highest non-toxic concentration of IFN-α used in this study) the viral titer reduction was 2.0, 2.9 and 3.6 $\log_{10}$ at an m.o.i. of 1.0, 0.1 and 0.01, respectively.

Table 3
Inhibitory effect of IFN-α on HAV replication in PLC/PRF/5 cells at 3 multiplicities of infection [a]

m.o.i.	Virus titer reduction ($\log_{10}$ $TCID_{50}$/ml) [b]			
	IFN-α concentration (IU/ml)			
	10	100	1000	10,000
1.0	0.1	0.6	1.0	2.0
0.1	0.2	0.7	1.5	2.9
0.01	0.7	1.4	2.5	3.6

[a] PLC/PRF/5 cells were infected with HAV at the appropriate m.o.i. in the presence of IFN-α and further incubated at 32°C for 12 days. On day 12, antiviral activity was evaluated by the determination of the infectious titer in cell culture.
[b] Means of quadruplicate experiments.

3.3. Effect of time of addition of IFN-α on virus yield from single-round replication

The effect of time of addition of 10,000 IU/ml of IFN-α on HAV replication is shown in Table 4. The pretreatment of cells with IFN-α before virus inoculation induced a significant decrease (t-test; $P = 0.05$) in infectious virus titer (0.9 $\log_{10}$). The greatest inhibition of HAV replication (2.0 $\log_{10}$) was observed when IFN-α was added 24 h before virus inoculation and incubated with cells for a single cycle of replication (pretreatment + post-treatment).

When IFN-α was added immediately after HAV adsorption (post-treatment), the virus titer was reduced by 1.6 $\log_{10}$. The antiviral activity was not significantly decreased (t-test, $P > 0.05$) when IFN-α was added 18 h after adsorption; the reduction of the virus titer was still 1.4 $\log_{10}$. The inhibitory effect of IFN-α on HAV replication was weakened, but still significant (t-test, $P = 0.05$), when the drug was added at 24, 36 or 48 h after adsorption. The viral titer reduction was 1.2, 0.7 and 0.5, respectively.

Table 4
Effect of time addition of IFN-α on HAV infectivity [a]

Duration of treatment (h) [b]	Infectious HAV titer (10^3 $TCID_{50}$/ml) [c]	Virus titer reduction ($\log_{10}$ $TCID_{50}$/ml)
Pretreatment [d]		
−24 → 0	113 ± 16	0.9
Pretreatment + post-treatment [e]		
−24 → +96	9 ± 2	2.0
Post-treatment [f]		
+0 → +96	23 ± 9	1.6
+6 → +96	25 ± 7	1.6
+12 → +96	26 ± 8	1.5
+18 → +96	33 ± 10	1.4
+24 → +96	57 ± 8	1.2
+36 → +96	165 ± 15	0.7
+48 → +96	268 ± 84	0.5
+60 → +96	450 ± 125	0.3
Control [g]	899 ± 170	–

[a] PLC/PRF/5 cells were infected at an m.o.i. of 1.0. The titers of infectious virus in cell samples were determined 96 h after viral inoculation.
[b] IFN-α, at the concentration of 10^4 IU/ml, was added before (−) or after (+) the inoculation of virus.
[c] Values are means ± S.D. of triplicate experiments.
[d] Cells were pretreated with IFN-α for 24 h at 32°C. The cells were then washed, infected and incubated in drug-free maintenance medium for 96 h.
[e] Cells were pretreated [d] then post-treated [f] with IFN-α.
[f] IFN-α was added at various times after the adsorption and the infected cells were incubated with the drug until 96 h postinfection.
[g] Virus-infected, non-drug-treated cells.

Table 5
Treatment of HAV-infected PLC/PRF/5 cells by IFN-α after a single cycle of virus replication [a]

Days after infection	Infectious HAV titer ($\log_{10}$ $TCID_{50}$/ml) [b]		
	IFN-α concentration (IU/ml)		
	0	1000	10,000
9	6.3	4.4	4.1
15	5.9	4.2	3.9
20	6.1	4.0	4.0

[a] PLC/PRF/5 cells were infected at an m.o.i. of 1.0 and incubated at 32°C for 4 days. At 96 h postinfection, a maximum infectious HAV titer of $10^{6.3}$ $TCID_{50}$/ml was achieved. The cells were then treated with IFN-α until the indicated time. Antiviral activity was evaluated by the determination of the infectious titer in cell culture.
[b] Data represent the means of triplicate determinations.

3.4. Antiviral activity of IFN-α in HAV-infected PLC / PRF / 5 cells after a single cycle of virus replication

Four days after infection at an m.o.i. of 1.0, when a maximum infectious HAV titer of $10^{6.3}$ $TCID_{50}$/ml was reached, cells were treated by 1000 or 10,000 IU/ml of IFN-α for different times. As shown in Table 5, virus yield was reduced about 100-fold in cells treated with IFN-α at the different times studied in the experiment (9, 15 or 20 days postinfection).

3.5. Measurements of 2',5'-oligoadenylate synthetase activity

In order to explore whether IFN-α exhibits its antiviral activity through induction of 2',5'-AS, a potent inhibitor of viral replication (Finter et al., 1991), we investigated 2',5'-AS activity in infected or non-infected cells (Table 6). In both cases, a dose-dependent activation of 2',5'-AS was observed (one-way ANOVA, $P < 0.05$). The 2',5'-AS

Table 6
2',5'-oligoadenylate synthetase activity in PLC/PRF/5 cells after treatment by IFN-α [a].

IFN-α (IU/ml)	Virus titer reduction ($\log_{10}$ $TCID_{50}$/ml)	Adenylate synthetase activity (2',5'-AS U) [b]			
		Non-infected cells [c]	n	HAV-infected cells [d]	n
0	–	0.05 ± 0.01	1 [e]	0.04 ± 0.01	1 [e]
100	0.6	0.74 ± 0.12	15	0.77 ± 0.07	19
1000	1.3	13.65 ± 3.18	273	13.50 ± 0.85	338
10,000	1.9	23.25 ± 3.18	465	18.14 ± 0.86	454

[a] PLC/PRF/5 cells were treated with IFN-α for 96 h. Then 2',5'-AS was assayed.
[b] One enzyme unit = 1 nmol AMP residues incorporated into 2',5'-oligoadenylates per minute at 37°C.
[c] Non-infected PLC/PRF/5 cells were incubated at 32°C for 96 h in the presence of different concentrations of IFN-α.
[d] PLC/PRF/5 cells were infected at an m.o.i. of 1.0 and incubated at 32°C for 96 h in the presence of different concentrations of IFN-α.
[e] Fold increase 2',5' activity: 2',5'-AS activity in IFN-α-treated cells/2',5'-AS activity in non-treated cells.

Table 7
Antiviral activity of IFN-α in combination with glycyrrhizin [a]

Glycyrrhizin concentration (μg/ml)	Virus titer reduction ($\log_{10}$ $TCID_{50}$/ml) [b]				Synergistic interaction [d] ($\log_{10}$ $TCID_{50}$/ml)
	GL alone	IFN-α alone	Both drugs	Enhancement of IFN-α activity [c]	
250	0.4	1.0	1.7	+0.7	+0.3
500	0.6	0.9	2.5	+1.6	+1.0
1000	1.0	1.1	3.1	+2.0	+1.0

[a] PLC/PRF/5 cells were infected with HAV at an m.o.i. of 1.0. Immediately after the adsorption, cells were treated with IFN-α (1000 IU/ml), glycyrrhizin (250, 500 or 1000 μg/ml) or combinations of these drugs and further incubated at 32°C for 12 days. On day 12, antiviral activity was evaluated by the determination of the infectious titer in cell culture. Viability was determined by Trypan blue exclusion. No toxic effect was observed (t-test; $P > 0.05$).
[b] Means of triplicate experiments.
[c] Enhancement of IFN-α activity compared with that of IFN-α alone: values are the additional decrement in virus yield with the drug combination compared with the effect of IFN-α alone.
[d] Additional decrement in virus yield with the drug combination beyond the algebraic sum of the decreases observed with either drug alone.

activity was about 0.05 unit/10^7 cells in non-treated cells. HAV infection by itself did not induce an increase in 2',5'-AS activity. This activity was increased about 15-, 300- and 460-fold in cells treated with 100, 1000 and 10,000 IU/ml, respectively. In parallel, a concentration-dependent reduction in HAV infectivity was observed.

3.6. Antiviral activity of IFN-α in combination with glycyrrhizin

The combination of IFN-α (1000 IU/ml) with 250, 500 or 1000 μg/ml of glycyrrhizin showed no significant cytotoxic effect (t-test; $P > 0.05$) for the 12-day cell exposure experiments.

As shown in Table 7, virus yield was reduced about 10-fold in cells treated with 1000 IU/ml of IFN-α. In cells treated with a combination of IFN-α (1000 IU/ml) and 250, 500 or 1000 μg/ml of glycyrrhizin, an average reduction of, respectively, 50-, 300- or 1000-fold in the yield of infectious virus was achieved. It was found that the combination of IFN-α and glycyrrhizin inhibited HAV replication in a synergistic manner, as shown by the additional reduction in virus yield compared with that from the sum of effects observed with each drug alone.

4. Discussion

In this study we demonstrated that IFN-α inhibited HAV replication in PLC/PRF/5 cells. IFN-α exerts a dose-dependent inhibitory effect on both virus-antigen expression and virus yield. The m.o.i. was found to have a substantial influence on the antiviral activity: a lower virus input m.o.i. increased antiviral activity. IFN-α also proved to be highly selective in its antiviral action: at an m.o.i. of 0.01, the concentration required to

inhibit virus replication was only 10 IU/ml, while the cytotoxic concentration (CD_{50}) for the host cells was above 10^4 IU/ml.

The effect of IFN-α was studied under different conditions. We showed that IFN-α had a prophylactic effect: the pretreatment of cells with IFN-α induced an antiviral state. IFN-α also showed a significant antiviral activity when it was added after infection. IFN-α still proved effective when it is added up to 48 h after the infection in a 96-h single cycle replication. Moreover IFN-α significantly reduced virus yield in HAV-infected cells when it was added after the first cycle of virus replication.

We observed an important increase in 2′,5′-AS activity in the IFN-treated human liver cells. 2′,5′-AS is an enzyme the synthesis of which is induced after binding of IFN to receptor sites and which often forms the basis of antiviral activity of IFN (Chebath et al., 1987). 2′,5′-AS acts in the presence of double-stranded RNA and synthesizes 2′,5′-adenylate oligomers (2′,5′-oligo A). The 2′,5′-oligo A then activates a ribonuclease, RNase L, which cleaves viral single-stranded RNA (Lengyel, 1982). Thus antiviral activity of IFN-α against HAV could be attributed, at least in part, to the induction of this enzyme in the PLC/PRF/5 cells.

IFN-α is now used for the treatment of two forms of viral hepatitis: chronic hepatitis B and C (Hoofnagle et al., 1986; Perillo et al., 1990). Partial studies showed that IFN-α therapy was also beneficial for patients with acute hepatitis C (Alberti et al., 1990; Chemello et al., 1992; Viladomiu et al., 1992; Yamamoto et al., 1992). IFN therapy would be useful for suppressing viral replication and quickly improving the ALT level (Hagiwara et al., 1992).

The efficacy of IFN-β to eliminate persistent hepatitis A infection in fibroblast culture has previously been described (Vallbracht et al., 1984). Moreover, a preliminary clinical study showed the efficacy of this type of IFN for fulminant and subfulminant viral hepatitis caused by HAV, although the therapeutic effects might be related to a suboptimal production of endogenous IFN in the patient. Administration of IFN-β to 3 patients with fulminant hepatic failure caused by hepatitis A and 1 patient with acute severe hepatitis A accompanying protracted liver failure induced a significant improvement of liver function and survival of all the patients (Yoshiba et al., 1994). In hepatitis A, viral antigen is first detected in the liver parenchyma of experimentally infected primates 1–2 weeks after intravenous inoculation and may persist there throughout the acute phase (Mathiesen et al., 1978). The detection of negative-strand HAV RNA just before peak ALT levels is consistent with active replication (Taylor et al., 1992). Clinical illness commonly lasts for about 4 weeks, but in 20% a relapse in liver inflammation may occur and symptoms may persist for up to 6 months (Hadler, 1991). Relapsing form is associated with a continuing viremia as well as excretion of virus in stools during the relapse phase. The pathogenesis probably involves an interaction between persistent viral infection and immune mechanisms responding to the continuing antigenic stimulation (Glikson et al., 1992). In fulminant hepatitis A, the detection of HAV-positive cells in human liver is strong evidence for the presence of active viral replication (Fagan et al., 1990). In such cases of severe or long-lasting forms of HAV infection, IFN therapy should be considered. Based on the in vitro efficacy of IFN-α to inhibit HAV replication even in persistently HAV-infected cell cultures, IFN-treatment could be effective in these severe forms. According to the results obtained in this study,

a combination of IFN-α and glycyrrhizin, an other effective anti-HAV substances (Crance et al., 1994a), could also be considered.

The results obtained in the present study on the inhibitory effect of IFN-α on HAV replication in vitro as well as its therapeutic activities already observed in patients with chronic and acute viral hepatitis suggest that recombinant IFN-α should be further evaluated for its efficacy in the treatment of severe forms of hepatitis A in adults.

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