

Decrease of Interferon-Induced 2',5'-Oligoadenylate Synthetase Activity in Cirrhotic Rat Liver

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Abstract

We evaluated the effect of hepatic fibrosis on the induction of hepatic 2',5'-oligoadenylate synthetase (2-5AS) by interferon (IFN) in a rat model of liver cirrhosis, induced with thioacetamide. Although there was no difference in serum 2-5AS activity between the control and cirrhotic rats given murine IFN, 2-5AS activity in the liver homogenates of cirrhotic rats was significantly lower than in the controls (105 ± 18.5 vs. 171 ± 10.2 pmol/ μ g, $p < 0.01$). These results suggested that hepatic fibrosis attenuated the effect of IFN and one of the reasons for this may be the decreased induction of hepatic 2-5AS activity after IFN administration in the presence of a cirrhotic liver.

Introduction

The efficacy of interferon (IFN) in the treatment of chronic hepatitis C depends upon 3 major factors: (a) the serum level of hepatitis C virus (HCV) RNA^{1,2}), (b) the HCV genotype^{3,4}), and (c) the extent of hepatic fibrosis^{5,6}). IFN is effective in only 15% of cirrhotic patients with chronic hepatitis C, much less than the 30-40% in those without cirrhosis⁷). These observations prompted us to examine whether hepatic fibrosis decreases the level of 2',5'-oligoadenylate synthetase activity (2-5AS) induced by IFN administration. To evaluate the relationship between 2-5AS activity and hepatic fibrosis we prepared a rat model of liver cirrhosis by intraperitoneally injecting thioacetamide and measured hepatic and serum activity of 2-5AS after IFN administration.

Materials and Methods

a) Production of liver cirrhosis in rats

Male Wistar rats (Kyudo Inc., Japan; average weight 170 g) were maintained at 23°C and on a 12-hour light-dark cycle. They had free access to a commercial balanced stock diet (CE-2; Clea Japan Inc., Japan) and to water.

We induced liver cirrhosis by the method of Zimmermann et al.⁸). Briefly, rats were intraperitoneally injected with thioacetamide, 20 mg/100 g body weight (BW) dissolved in 0.5 ml saline, twice a week for 16 weeks (LC group). Control rats were given saline, 0.5 ml/100 g BW, using the same administration regimen as the LC group (saline group). Experiments were started 2 weeks after the final injection of thioacetamide or saline.

b) Reactivity of rats and mice to IFN

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The murine IFN (IFN- α/β) was a gift from Otsuka Pharmaceuticals Co., Ltd. (Hayashibara Biochemical Research Center, Japan), and the human lymphoblastoid interferon (HLBI) was a gift from Sumitomo Pharmaceuticals Co., Ltd. (Japan). Rats were given either murine IFN or HLBI to investigate serum 2-5AS activity. The dose of IFN given to the rats was equivalent to the dose given to chronic hepatitis C patients¹⁾. Murine IFN, 1×10^4 IU or 5×10^4 IU per 100 g BW (total dose: 0.2 ml/body), was given to the untreated rats via the left facies lateralis cruris. Saline, 0.2 ml, was administered via the same route to 2 rats in the untreated group. HLBI 5×10^4 IU/100 g BW, IM, was administered to 2 rats in the untreated group, and 3 BALB/c mice (Japan SLC Inc., Japan) were also given murine IFN, 1×10^4 IU or 5×10^4 IU per 100 g BW, IM, via the left facies lateralis cruris.

To measure 2-5AS activity, blood was collected from the caudal vein of the rats or the inferior vena cava of the mice before, and 12 and 24 hours after administration. Following separation by centrifugation at 2500 rpm at 4°C for 10 minutes, the sera were frozen and stored at -20°C until 2-5AS activity assay.

c) Serum 2-5AS activity in the control and cirrhotic rats

Murine IFN, 1×10^4 IU or 5×10^4 IU per 100 g BW (total dose: 0.2 ml/body), or saline was given to 6 cirrhotic rats (2 rats from each group).

Murine IFN or saline was also given to 2 rats from the control group (total 6 rats) at the same doses via the same route. Serum was collected for 2-5AS activity assay before, and 12 and 24 hours after administration.

d) 2-5AS activity in rat liver

Murine IFN, 5×10^4 IU/100 g, BW, or saline, 0.2 ml/body, was injected intramuscularly via the left facies lateralis cruris to 6 rats in each group (LC and control). Serum 2-5AS activity was measured before and 24 hours after administration. The animals were then anesthetized by intraperitoneal injection with 0.1 ml pentobarbital per 100 g BW. Laparotomy was performed in the midline, the portal vein was ablated, and exsanguination was performed by perfusion with Ca⁺⁺- and Mg⁺⁺-free phosphate-buffered saline via the portal vein followed by resection of the liver. A part of each resected liver was sliced and added to lysis buffer [10 mM Hepes (pH 7.5), 20% glycerol, 0.3 mM EDTA, 3 mM (CH₃COO)₂Mg, 50 mM KCl, 1 mM dithioerythritol, and 0.5% nonidet P-40] and allowed to stand for 15 minutes at 4°C. The preparations were centrifuged at 10,000 rpm at 4°C for 15 minutes, then homogenized in a stainless steel homogenizer (Tokyo Rikakidai Co., Ltd., Tokyo, Japan) at 4°C. The protein content and 2-5AS activity of the supernatants were measured.

e) Quantification of 2-5AS activity

The activity of 2-5AS, an intracellular enzyme induced by IFN, was determined with a radioimmunoassay kit (Eiken, Japan).

f) Quantification of protein concentration

The protein concentration of liver homogenates was measured with a DC protein assay kit (BIO RAD, Japan). The reaction in this assay is similar to the well-documented Lowry assay⁹⁾.

g) Liver histology

All histological evaluations were performed blindly by two independent pathologists.

h) Statistical analysis

The results are expressed as means \pm SD. Statistical analysis was performed by the Mann-Whitney U test; over 95% confidence ($p < 0.05$) was considered significant.

Results

Light microscopic examination showed that all rats given thioacetamide had pseudolobules in their livers, similar to the findings in human liver cirrhosis (Fig. 1).

Fig. 1 Histology of the cirrhotic rat liver. Rats were given thioacetamide for 16 weeks (See Materials and Methods). Many regenerating nodules of various sizes are visible. Hematoxylin-eosin stain. Original magnification $\times 66$.

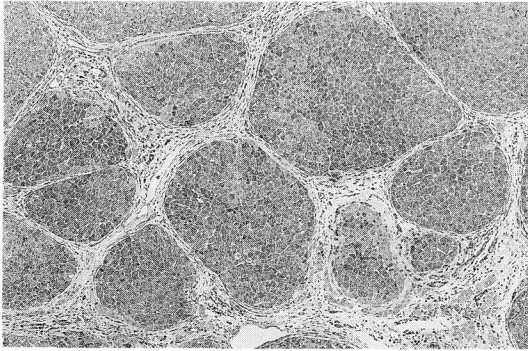


Fig. 2 Time course of serum 2-5AS activity in untreated mice and rats injected with IFN. Serum 2-5AS activity was measured before, and 12 and 24 hours after administration of IFN or saline. The mean levels before the administration were set equal to 100%. Bars: means \pm SD. \blacktriangle : Murine IFN, 5×10^4 IU/100 g BW; \blacksquare : Murine IFN, 1×10^4 IU/100 g BW; \bullet : 0.2 ml saline; \circ : HLBI, 5×10^4 IU/100 g BW.

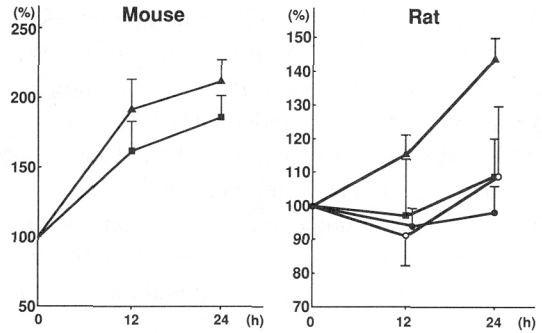


Fig. 3 Time course of serum 2-5AS activity in control and LC rats after IFN injection. Serum 2-5AS activity was measured before, and 12 and 24 hours after administration of IFN or saline. The mean levels before administration were set equal to 100%. Bars: means \pm SD. \blacktriangle : Murine IFN, 5×10^4 IU/100 g BW; \blacksquare : Murine IFN, 1×10^4 IU/100 g BW; \bullet : 0.2 ml saline.

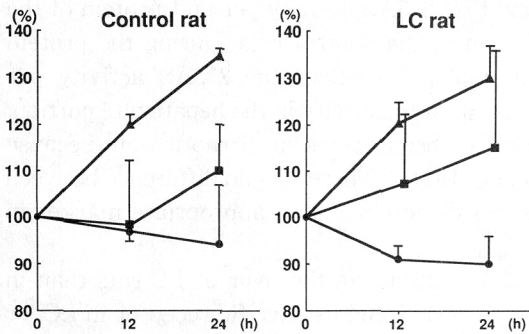
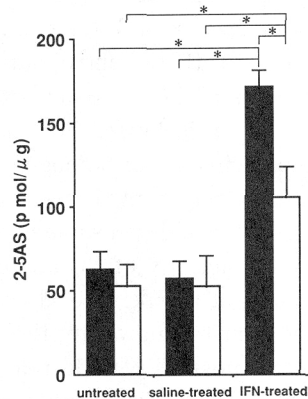


Fig. 4 The 2-5AS activity in liver homogenates. The 2-5AS activity in liver homogenates was measured 24 hours after administration in control and LC rats given murine IFN, 5×10^4 IU/100 g BW, or 0.2 ml saline. \blacksquare : control rats, \square : LC rats. Bars: means \pm SD. * $P < 0.01$.



a) Serum 2-5AS activity in untreated mice and rats

BALB/c mice given murine IFN at a dose of 1×10^4 IU/100 g BW had significantly higher serum 2-5AS activity 12 and 24 hours after administration than before (Fig. 2, left). Administration of the 5×10^4 IU/100 g BW dose further increased serum 2-5AS activity.

Murine IFN at a dose of 5×10^4 IU/100 g BW significantly increased serum 2-5AS activity in untreated rats, but the 1×10^4 IU/100 g BW dose did not significantly increase serum 2-5AS activity even 24 hours after administration (Fig. 2, right). There was no change in 2-5AS activity in the serum

of rats given only saline, nor was there any significant change 12 and 24 hours after administration in untreated rats given HLBI at a dose of 5×10^4 IU/100 g BW (Fig. 2, right).

b) Serum 2-5AS activity in control and cirrhotic rats

There was no significant increase in serum 2-5AS activity in control or LC rats given murine IFN at a dose of 1×10^4 IU/100 g BW (Fig. 3). However, there were significant increases in both control and LC rats at a dose of 5×10^4 IU/100 g BW 12 and 24 hours after administration. IFN increased serum 2-5As activity by $30.0 \pm 10.0\%$ in LC rats and by $30.0 \pm 9.0\%$ in control rats, and there was no significant difference between the groups.

c) 2-5AS activity in the liver homogenates of control and cirrhotic rats

The 2-5AS activity in liver homogenates from LC rats was significantly lower ($p < 0.01$) than in the control rats (LC rats, 105 ± 18.5 pmol/ μ g; control rats, 171 ± 10.2 pmol/ μ g) (Fig. 4). The 2-5AS activity of liver homogenates from the control and LC rats increased significantly in the murine IFN-treated group as compared to the untreated and saline-treated groups ($p < 0.01$ in both). We found no significant difference between the 2-5AS activity of liver homogenates from control and LC rats which were untreated or given only saline (Fig. 4).

There was no increase in 2-5AS activity in liver homogenates from untreated rats given HLBI (64.3 ± 24.0 pmol/ μ g), and thus no cross reactivity between species.

Discussion

The thioactamide-induced LC rats in the present study had regenerative nodules with fibrous septa and portal hypertension, similar to the findings in human liver cirrhosis^{8,10}. We evaluated the induction of an antiviral state in the liver by measuring liver 2-5AS activity after IFN administration. IFNs are species-specific, as previously reported and confirmed in this study, but murine IFN shows cross-reactivity to rat cells¹¹. We observed elevated 2-5AS activity in rats given murine IFN, but not in the controls given saline (Fig. 2). A much greater induction of serum 2-5AS activity was observed at a higher IFN dose in both the control and LC rats. Based on these findings we gave murine IFN in a dose of 5×10^4 IU to control and LC rats and found that 2-5AS activity per unit protein of the liver homogenates of LC rats was significantly lower than in the control rats. During the protein assay we used a lysis buffer in which collagen is insoluble. Therefore, the 2-5AS activity per non-collagen protein of liver homogenates, even in LC rats, showed activity in the hepatocyte portion. Decreased 2-5AS may contribute to the antiviral nature of hepatocytes in cirrhotic rats because induction of 2-5AS is indispensable in mediating the effect of IFN¹². There was no difference between the serum 2-5AS activity of LC and control rats, thus 2-5AS activity is an appropriate marker in affected organs, but not peripheral compartments¹¹.

It is important to know why IFN induced lower 2-5AS activity in the liver of LC rats than in normal rats. One possibility is decreased association between IFN and its specific receptor in LC, or in other words, a decreased concentration of IFN at the target site. Total hepatic blood flow is decreased in the cirrhotic liver^{13)~15}, and histologically, the hepatic sinusoids exhibit capillarization¹⁶. These two major changes may decrease the binding of IFN to its receptor on the hepatocyte surface. It is not known if changes in receptors (decreases in number of receptors per hepatocyte, changes in their location on the hepatocyte, etc.) and changes in intracellular signals occur after binding to LC rat receptors.

In conclusion, we have demonstrated that IFN-induced 2-5AS activity in the liver of LC rats is significantly lower than in control rats. This finding suggests that hepatic fibrosis attenuates the effect of IFN, and one reason for this may be the decreased induction of hepatic 2-5AS activity after IFN administration in the presence of cirrhotic liver.

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ラット肝硬変モデルにおける抗ウイルス状態誘導能

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要 旨

C型慢性肝炎に対するインターフェロン(IFN)療法の効果は、肝組織の線維化の進展に伴い低下し、特に、肝硬変に対する著効率は極めて低い。そこで著者らは、肝組織の線維化の進展が、何故、IFNの治療効果を低下させる要因になるかを明らかにするために、ラット肝硬変モデルを用いて抗ウイルス状態の誘導能を検討した。抗ウイルス状態の誘導能は、IFNにより誘導される2',5'オリゴアデニル酸合成酵素活性(2-5AS)で評価した。

肝硬変ラットとコントロール・ラットにおいて、IFN投与後の血清2-5ASの変化には、差を認めなかったが、肝ホモジネート中の2-5ASは、肝硬変ラットにおいて有意に低かった(105±18.5vs. 171±10.2pmol/μg, p<0.01)。今回の実験結果から、肝組織の線維化が、IFNによる抗ウイルス状態の誘導能を低下させる要因になっている可能性が示唆され、また、このことが肝硬変においてIFNの治療効果が低い要因の一つと考えられた。