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Studies on the interference of ganciclovir to HCV liver fibrosis

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Abstract. – OBJECTIVE: To investigate the significance of the combined treatment with ganciclovir and interferon for patients with hepatitis C (HCV) liver fibrosis.

PATIENTS AND METHODS: We retrospectively summarize 86 patients with hepatitis C treated in our hospital from October 2013 to October 2015. 49 cases, considered as control group, received combined treatment with α-interferon and ribavirin; 37 cases, considered as observation group, received combined treatment with ganciclovir and interferon. The changes of liver fibrosis, viral replication and liver function of both groups were compared for two weeks and six months.

RESULTS: The levels of sera hyaluronic acid (HA), laminin (LN), type IV collagen (IVC) and type III procollagen (PIII NP) of both groups were reduced after treatment, and the observation group improved more significantly ($p < 0.05$). Compared to the rate of antigen-positive after treatment and HCV copy number before and after treatment, the differences were not statistically significant ($p > 0.05$). The level of alanine aminotransferase (ALT) of the control group increased after treatment, compared with that before. This was done along with the decrease of the level of albumin. By contrast, the level of ALT in the observation group was reduced and the level of albumin was increased compared with that before ($p < 0.05$).

CONCLUSIONS: Ganciclovir combined with interferon may further reduce the fibrosis process of patients with hepatitis C, and may improve liver function. The effect of antiviral was similar as ganciclovir combined with Interferon was comparatively good applied, safety and effectiveness.

Key Words:
Ganciclovir, Interferon, Ribavirin, Hepatitis C, Liver fibrosis.

Introduction

According to World Health Organization statistics, about 200 million people are infected with chronic hepatitis C (referred to as "HCV"). About 35% are found in China, among which, 10 to 20% of patients are at risk for cirrhosis and liver cancer[1]. As a positive strand RNA virus, with significant heterogeneity and a high degree of variability, Hepatitis C virus is easy to produce resistance to antiviral drugs[2]. In the current clinic, ganciclovir combined with Interferon is regarded as the standard treatment. It has 40 to 50% sustained viral response (SVR) to genotype 1, 70 to 80% to genotype 2 and genotype 3[3]. In China, we focus on genotypes 2 and 3. However, due to non-typical manifestations in the early clinic and poor management in incipient identification and intervention norms, it easily leads to poor treatment effect[4]. Liver fibrosis is a necessary stage for chronic active hepatitis progressing to cirrhosis. The processes of reverse liver fibrosis are of great significance to improve the antiviral effect and the prognosis[5]. Ganciclovir gives priority to inhibit viral DNA synthesis, which plays an important role in the treatment of cytomegalovirus infection[6]. Recently, we have seen the gradual expansion of the scope of ganciclovir application, such as herpes zoster virus, infectious mononucleosis syndrome, and viral encephalitis. Some studies have shown that[7], ganciclovir has interventional effect on the process of liver fibrosis and liver cirrhosis process caused by various kinds of liver viruses. But the exact mechanism is still unclear. The purpose of the current study was to analyze the clinical effect of ganciclovir combined with Interferon towards patients with hepatitis C liver fibrosis and provide new ideas for the prevention of hepatitis C.

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Patients and Methods

Patients

We retrospectively reviewed 86 cases of patients with hepatitis C who treated in our hospital for the first time from October 2013 to October 2015. They were all detected and diagnosed by serology and virology. Inclusion criteria: (1) Aging from 18-70 years old; (2) Positive virus antigen, virus load ≥ 10^3 IU/ml; (3) Accepting antivirus scheme till the virus became negative and maintaining treatment for at least 3 months. Exclusion criteria: 1. Combined hepatitis B, cirrhosis, liver cancer and autoimmune hepatitis, etc. 2. Women during pregnant and breast-feed stage; (3) Multi-consolidated underlying disease, such as heart, lung, kidney and other organs dysfunction; (4) Poor compliances, incomplete clinical material, etc.

43 cases were divided into the control group and 37 in the observation group according to the different treatment methods. The control group adopted combined treatment with α-interferon and ribavirin, while the observation group adopted combined treatment with interferon and ganciclovir. 28 cases were male and 21 cases female in the control group; aging from 35-68 with an average age of (52.3 ± 14.2) years old; the course of disease was 1-3 months with and average course of (1.4 ± 0.8) months. 20 cases were male and 17 cases female in the observation group; aging from 36-69 with an average age of (53.4 ± 13.6) years old; the course of disease was 0.5-3.5 months with an average course of (1.5 ± 0.7) months. Compared gender, age and course of disease between two groups, the differences were not statistically significant ($p > 0.05$).

Methods

Patients of both groups were all given with symptomatic treatment, including liver protection, enzyme reduction and nutritional support. As well, exogenous albumin was supplemented when necessary, and virus, liver function, routine blood test and coagulation index were monitored regularly. α-interferon combined with ribavirin scheme: interferon was Anferon 5 million U. The doses of ribavirin were in accordance with the recommendations of the guide, weight: > 85 kg, recommended dose was 1200 mg/d, weight: 65-85 kg, recommended dose was 1000 mg/d, weight: < 65 kg. Recommended dose was 800 mg/d, but not less than 10.6 mg/kg. Continuously treated for 72 weeks and observed after the drug withdrawal for 24 weeks. Whether there was hemolytic anemia, renal failure, nausea, vomiting, coughing, erythar, hyperuricemia. Any other side effects were closely monitored during the treatment, and erythropoietin, folic acid tablets, vitamin B12 were supplemented when necessary, or stopped drug administration and observed.

Interferon combined with ganciclovir scheme: interferon dose was the same as above, ganciclovir dose referenced to the literature, there were prevention period and induction period, in prevention period, two intravenous injections a day with 5 mg/kg at a time, each injection time shall be more than 1h, lasting for 14-21 days; in holding period, intravenous injection was 6 mg/kg a day, five days a week or 5 mg/kg a day, seven days a week. Continuously treated for 72 weeks and observed after drug withdrawal for 24 weeks. Adverse reactions shall be monitored closely during the treatment.

Observation Index

Serum liver fibrosis before and after treatment was contrasted. These include hyaluronic acid (HA), laminin (LN), type IV collagen (IVC) and type III procollagen (PIII NP) levels, and viral replication includes antigen-positive and HCV copy number, liver function includes ALT and albumin level. Radioimmunoassay was used to test liver fibrosis index. Kits were purchased from Shanghai Haiyan Medical Biotechnology Center. Procedures were in strict accordance with the instruction; viral replication level used CMIA method and Abbott Architect 12000 full automatic chemiluminescence immunoassay system as well as matching reagent (Abbott, Abbott Park, IL, USA) to test serum antigen. Bio-Rad I cycler fluorescent quantitation PRC instrument was used to detect serum HCV copy number (Shanghai Shenggong Technology Co., Ltd., Shanghai, China). Liver function test used Hitachi automatic biochemical analyzer (Tokyo, Japan) inter-assay and intra-assay variation was less than 5%.

Statistical Analysis

SPSS20.0 software (SPSS Inc., Chicago, IL, USA) was used to carry out statistical analysis. Measurement data were expressed by the mean ± standard deviation (SD). Comparisons among groups were tested by independent sample *t*-test. Comparison within group was tested by paired *t*-test. Count data were expressed by the number of case or a percentage. Comparisons among groups were tested (corrected) by χ^2. $p < 0.05$ indicated that the differences were statistically significant.

Results

Comparison of Serum Liver Fibrosis Index

Sera HA, LN, IVC and PIII NP levels of both groups before treatment were compared. The differences were not statistically significant ($p > 0.05$). All the above mentioned parameters decreased after treatment. Furthermore, the observation group improved more significantly ($p < 0.05$) (Table I).

Comparison of Viral Replication Level

Antigen positive rate after treatment, and number of copy of HCV before and after treatment of both groups were not statistically significant ($p > 0.05$) (Table II).

Comparison of Liver Function Level

ALT and albumin levels of two groups before treatment were compared. The differences were not statistically significant ($p > 0.05$). The ALT level of the control group after treatment increased compared with that before treatment while albumin level decreased. As well, ALT level of the observation group decreased compared with that before treatment, while albumin increased. The differences of comparisons among groups or within group were statistically significant ($p > 0.05$) (Table III).

Discussion

The gold standard for diagnosis of liver fibrosis is liver biopsy tissue diagnosis, but it will cause liver damage and with many complications, which cannot be detected repeatedly as confirmed elsewhere[8,9]. Levels of serum fibrosis indexes HA, LN, IVC and PIII NP have a better consistency to the liver inflammation activity and fibrosis. They can be regarded as a diagnosis and extent assessment of liver fibrosis. Wherein HA and IVC are in a pathological stage from S1-S4, they have seen great increase successively, which was better than LN in terms of the early diagnosis of liver fibrosis. PIII NP has seen significant increase in succession in S2 period, which could be regarded as auxiliary diagnostic criteria in middle and advanced stages of liver fibrosis[10]. In addition, viral antigens, antibodies and viral load are closely related to the degree of liver inflammation and fibrosis[11].

The pathogenic mechanism of HVC is that the proliferation of HVC directly damages the structure and the function of the liver cells. This interfering with the synthesis of cell protein, disrupting the normal functions of transporting and metabolism of liver, resulting in the degeneration and necrosis of liver cells[12]. The toxicity reaction of an immune cell also plays an important role. Histology has found that a large number of CD8 + T cell infiltration, delayed hypersensitivity and specificity of cytotoxicity attacked target cells infected with HCV, resulting in liver injury[13]. Through the in-depth study of the hepatitis C virus, we have found that compared to the interferon treatment, the method of taking the HCV life cycle and important viral protein crystal structure as the specific target (including the NS3/4A serine protease, NS5A replication complex proteins, NS5B RNA-dependent polymerase, NS4B and NS3 helicase protein) could greatly improve patients' SVR. It could also shorten the treatment time, increase the tolerance, protect the liver function, and reduce the side effects of drugs. It is referred as direct antiviral (DAA).

Ganciclovir is a guanosine derivative. Similar to acyclovir, it is the first effective drug for human cytomegalovirus, which all, can suppress herpes virus[14]. Its concentration in the virus-infected cells may be 100 times higher than non-infected cells. The inhibition of viral replication is mainly in two ways: the first one is that its triphosphate (GTP)

Table I. Comparison of TAS, TOS and oxidative stress markers among the groups.

Group	HA (μg/ml)		LN (μg/ml)		IVC (μg/L)		PIII NP (μg/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	123.6 ± 32.6	82.3 ± 20.5	142.5 ± 42.3	90.3 ± 23.6	72.5 ± 23.8	44.6±12.3	92.7 ± 23.3	56.4 ± 16.9
Observation group	135.4 ± 35.7	56.7 ± 24.0	153.7 ± 45.7	62.4 ± 24.5	74.3 ± 25.6	21.7±15.2	93.6 ± 25.2	35.2 ± 12.3
t	0.235	5.637	0.216	5.649	0.423	5.724	0.326	5.928
p	0.765	0.032	0.769	0.030	0.638	0.026	0.638	0.021

Table II. Comparison of viral replication level.

Group	Case	Antigen-positive after treatment [case (%)]	HCV copy number before treatment (10^3 IU/ml)	HCV copy number after treatment (10^3 IU/ml)
Control group	49	6 (14.0)	2.2 ± 0.6	0.5 ± 0.1
Observation group	37	4 (10.8)	2.3 ± 0.5	0.8 ± 0.2
t		0.000	0.236	0.215
p		1.000	0.827	0.932

Table III. Comparison of liver function level.

Group	ALT (U/L)		Albumin (g/L)	
	Before treatment	After treatment	Before treatment	After treatment
Control group	72.6 ± 5.6	82.4 ± 8.3	32.6 ± 4.3	25.0 ± 5.3
Observation group	73.2 ± 5.5	25.7 ± 5.7	34.2 ± 4.2	43.6 ± 5.6
t	0.257	6.328	0.632	5.968
p	0.725	0.016	0.421	0.023

competitively inhibits the combination of deoxyguanosine triphosphate and DNA polymerase, thus inhibiting viral DNA polymerase[15]; the second one is that it directly incorporate viral DNA, which can inhibit DNA synthesis, and prevent the prolongation of viral strand of DNA. The effect can be enlarged by GTP accumulating in CMV infected cells[16]. In addition, the study found that ganciclovir's oral preparation could effectively inhibit the activity and replication of HBV virus, and ganciclovir is still sensitive to lamivudine-resistant mutants. Although HCV is an RNA virus, the clinical application has discovered that[17], ganciclovir still has a high response rate and inhibition rate towards HCV. The specific mechanism is still not clear. It may be related to the combination with interferon which improves the activity of interferon antiviral or the improvement of body's antiviral immunity[18].

We draw a conclusion from the study. The fact that the levels of serum HA, LN, IVC and PIII NP of both groups reduced, and that the observation group improved more significantly suggested that ganciclovir combined with interferon may have a better effect on reversing liver fibrosis[19]. Comparing the rate of antigen positive and the number of copy of HCV before and after treatment, lead to differences that were not statistically significant. This suggests that the effect of antiviral of ganciclovir combined with interferon was equivalent, and the function of reverse liver fibrosis may be irrelevant to the function of antiviral. After treatment, the levels of ALT of the control group increased, compared with that before treatment, albumin levels decreased while in the observation group, ALT levels reduced and the levels of albumin increased. The differences of comparisons within group and among groups were statistically significant and suggest that in improving liver function effect, ganciclovir combined with interferon are evident. This finding also showed that the reversal of liver fibrosis may be related to the improvement of liver function[20,21].

Conclusions

Ganciclovir combined with interferon may further reduce the fibrosis process of patients with hepatitis C, improve liver function. The effect of antiviral is the same as ganciclovir combined with interferon, which has good application safety and effectiveness.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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