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Interferon-alfa treatment may reduce the occurrence of liver carcinogenesis in patients with chronic viral hepatitis

Hepatocellular carcinoma (HCC) is one of the 10 most common tumors in the world (14). Hepatitis viruses, particulary the hepatitis B virus (HBV) and the hepatitis C virus (HCV), are the major environmental causes of human hepatocellular carcinoma (HCC) world-wide as they induce chronic liver disease and cirrhotic transformation of the liver (1, 7). These two major aethiological factors have been definitively incriminated in the pathogenesis of HCC. Approximately, 300 million people are chronic carriers of HBV and about one percent of the world's population is infected with HCV. HCC for most patients is a terminal complication of chronic inflammatory and fibrotic liver diseases. With regrettably few exceptions, the treatment of this neoplasm is largely palliative, and long-term survival is rare. Over one million patients die of HCC annually (13).

The molecular mechanism of HBV and HCV-related liver carcinogenesis remains debated. There is evidence for biological effects of proteins of both viruses, which can directly modulate major transduction cellular signals controlling cell proliferation, differentiation and viability, yet still the pathogenesis of HCC is poorly understood (10).

A current discussion focuses on the question whether interferon alfa administered to patients with chronic liver disease (with or without biochemical or virologic response) delays or prevents cancer of the liver. The literature suggests that interferon therapy may prevent hepatocellular carcinoma in patients with cirrhosis, particularly those with HCV (2, 3, 9, 12, 15).

Serologic tests (for example alpha- fetoprotein (AFP) and ultrasonography are used in HCC screening (14). AFP is α_1- globulin secreted by fetal hepatocytes and in small amount by other cells of the fetal gastrointestinal tract. Physiologically, in human adults an increased AFP level is present in the serum of pregnant women. In pathology, measurements of alpha-fetoprotein (AFP) are an important tool in the care and management of patients with benign and malignant hepatic disorders. Elevation of serum AFP in benign hepatic diseases (acute and chronic viral hepatitis, toxic liver injury) is associated with small, transient increases in serum (4, 5, 6, 8). Therefore, quantification of serum AFP has been widely used as a diagnostic marker for HCC. The concentration of AFP correlates with the tumor's size and after an effective surgical procedure it should decrease or even normalize. Measurements of serum AFP levels have also been used in screening the populations at high risk of human HCC such as those with cirrhosis or carriers of HBV and HCV viruses (5, 8).

To determine the influence of interferon-alfa (IFN-α) treatment on the serum marker of hepatocarcinogenesis levels in patients (pts) with HBV and HCV-related chronic liver disease, we examined and compared serum AFP levels before, after therapy and during 4–7 years' follow up.

MATERIAL AND METHODS

Characteristics of the patients selected for the study. Thirty seven patients with chronic hepatitis B and C were selected for IFN α treatment. The patients were divided into the following groups: I studied group – 16 patients with chronic hepatitis B (CH B), including 7 women and 3 men, age range – 18–69 years; II studied group – 21 patients with chronic hepatitis C (CH C), including 13 women and 8 men, age range – 18–62 years. The characteristics of the patients of both groups are presented in Table 1.

Table 1. Characteristics of patients with chronic hepatitis B and C before interferon–α therapy

	Chronic hepatitis B (n= 16)	Chronic hepatitis C (n= 21)
Age* (years)	43 ± 14	42 ± 12
Range of age (years)	18–66	18–62
Sex: female	7	13
male	9	8
Source of infection:		
- medical procedures	13	20
- family contact	1	0
- professional exposure	1	0
- sexual contact	1	0
- unknown	0	1
Acute hepatitis in anamnesis	10	4
Duration of infection (years)*	4 ± 4	6 ± 6
(range)	(1–18)	(1–16)

* All data are expressed as mean ± SD

The patients treated with IFN-α in HBV and HCV groups did not significantly differ with regard to changes in the serum levels of aminotransferases, alkaline phosphatase, γ– glutamyl-transpeptidase, bilirubin, albumin, γ–globulin, platelets and prothrombin time. Laboratory findings are shown in Table 2.

Interferon α–2b (IFN α) (Intron A, Schering–Plough) was administrated subcutaneously, 3 x a week in the dose of 9 or 15MU/week. Since some patients developed marrow suppression, the treatment was periodically discontinued or the dose reduced. The treatment duration was: 16 weeks in CH B patients and 24–52 weeks in CH C patients. After the treatment the patients were followed up once a month and, the final efficacy of therapy was evaluated after one year. The serum AFP levels were checked every 6 months for 4–7 years following the treatment.

Laboratory tests. At the beginning of the treatment morphology with absolute granulocyte and thrombocyte count was performed after each IFN α injection. Then these tests as well as alanine and aspartate aminotransferases (ALT, ASP), alkaline phosphatase, gamma-glutamyltranspeptidase, bilirubin determinations were performed once a week.

The levels of total protein, protein electrophoresis, coagulation parameters (prothrombin time), and renal efficiency (creatinine, urea) were evaluated every month. HBV-DNA (using Digene Hybrid Capture System – Murex Diagnostica GmbH, Burgwedel) and HCV RNA (RT PCR) were determined before, in the middle and directly after the treatment, then a half and one year after. The serum AFP levels were measured by a RIA – AFP – PROP/J^{125}, MJ^{137}/ test.

Table 2. Laboratory findings in patients with chronic hepatitis B and C before Interferon–α therapy*

	Chronic hepatitis B (n= 16)		Chronic hepatitis C (n= 21)	
	mean	range	mean	range
ALT (U/l)	294 ± 206	57–798	160±109	61–578
AST (U/l)	212 ± 200	27–990	111 ± 63	48–320
AP (U/l)	114 ± 51	48–321	90 ± 28	45–169
GTP (U/l)	124 ± 153	14–820	57 ± 44	10–185
Bilirubin (umol/l)	15 ± 12	5–72	15 ± 12	7–60
Albumins (g/dl)	4.26 ± 0.48	3.46–5.97	4.40 ± 0.40	3.50–5.56
Gammaglobulins (g/dl)	1.60 ± 0.59	0.90–4.16	1.40 ± 0.50	0.14–2.57
Platelets (G/l)	153 ± 37	82–224	187 ± 49	101–333
Prothrombin time (s)	16 ± 2	12–20	15± 2	12–20

Normal range: alanine aminotransferase (ALT)–5– 40 U/l, aspartate aminotransferase (AST)– 5– 40 U/l, alkaline phosphatase (AP)–35–125 U/l, gamma–glutamyltranspeptidase (GTP)– 10–75 U/l, bilirubin–5–17 umol/l, albumins–3.5–5.5 g/dl, gammaglobulins– 0.91– 1.48 g/dl, platelets–100– 350 G/l, prothrombin time–12–16 s.

* All data are expressed as mean ± SD

Statistics. Statistical analysis was performed using the unpaired Student's t-test, the Mann-Whitney U-test, the χ^2 test with Fisher's correction. The significance level was set at $p< 0.05$. The results are expressed as means ± SD. The statistical analysis was performed using SPSS PC+ software.

RESULTS

The effectiveness of IFN α treatment was evaluated in both groups directly after and one year after therapy. The lack of serum HBV DNA or HCV RNA one year after the treatment was assumed to be a complete and sustained response. Twenty out of 37 patients (54%) achieved a complete and sustained response to treatment (11/16 from HBV group and 9/21 from HCV group) and 17 showed no such response.

The baseline serum AFP level was increased in 26 out of 37 patients (70%) (14/21 from HCV group; 12/16 from HBV group). After the 4–7 years' follow-up it remained increased only in 2 out of 37 patients (5%) (1 from HBV and 1 from HCV group). The AFP values significantly decreased after IFN α treatment (17.58 ± 19.09 IU/ml vs. 7.95 ± 21.78 IU/ml; p, 0.05; normal range 0–5 IU/ml) in both HBV and HCV, responder and non-responder groups. These results are presented in Table 3.

HCC developed after the 5 years' follow-up in one patient, who was a non-responder female with chronic HBV infection, with the baseline AFP level – 24.4 IU/ml and the follow-up AFP level (5 years after the treatment) – 29.42 IU/ml.

Table 3. Serum α–fetoprotein level in patients with chronic hepatitis B and C*

Group of patients	Serum α–fetoprotein level (IU/ml)		P
	before IFN α	after IFN α	
All (n=37)	17.58 ± 19.09	7.95 ± 21.78	< 0.05
Responders (n=20)	13.14 ± 11.66	4.35 ± 3.36	= 0.001
Non–responders (n=17)	26.09 ± 25.96	13.92 ± 35.31	< 0.05

* All data are expressed as mean ± SD; IFN α–interferon α

DISCUSSION

The treatment of chronic hepatitis B and C remains difficult despite recent progress in this field. Due to prophylactic vaccination, HBV infections can be prevented and the whole situation seems to be under control. On the other hand, the problem of HCV infections, whose diagnosis by specific tests became possible only in 1989, increases. There are about 100 million HCV carriers world-wide (1,7,14). It should be stressed that these infections are statistically undetectable as the majority of them are asymptomatic. So the reported numbers show only "the tip of the iceberg".

The use of IFN α in the treatment of hepatotropic virus infections gave us hope to fight the disease. At present, there is no doubt that the drug administrated in chronic hepatitis inhibits its progression in some patients (2, 3, 9, 12, 11, 15). However, the long- term effects remain highly unsatisfactory.

Strict selection criteria were used in the group of patients analyzed by us. Thanks to them 91% of patients completed their treatment. Similar results were obtained by other authors who used similar selection procedures (11). All patients with CH B and CH C selected for interferon therapy had elevated aminotransferases activity. As the literature data show in some CH B patients, HBsAg, HBeAg and HBV DNA are likely to be detected with normal or slightly increased levels of hepatic enzymes. Such patients respond poorly or do not respond to IFN α treatment. They should not be selected for therapy but constantly followed up (11).

In our population, the mean ALT activity before treatment was lower in patients with CH C compared with patients with CH B. However, even normal activity of this enzyme does not objectively reflect the degree of liver damage in chronic HCV infections. Contrary to patients with CH B, group with CH C and low aminotransferases activity better respond to IFN α treatment (11). The majority of patients treated in our Department received interferon α in the dose of 5 MU 3 times a week. Some patients had their doses modified as the symptoms of marrow suppression developed, i.e. decreased absolute neutrophil and thrombocyte counts. The efficacy of IFN α therapy in the examined population was not very satisfactory (54%), particularly in HCV patients (42%), in whom also the number of recurrences within one year following the treatment was higher (compared to the group with HBV infection).

At present, the studies are carried out to explain the role of IFN α for HCC prevention in patients with HBV and HCV-related chronic liver disease. Our results show that IFN therapy could diminish the risk of liver carcinogenesis in these patients because it significantly decreases the serum AFP level. Its beneficial effect was observed in responder as well as non-responder groups. During the follow-up, HCC was detected in one patient 5 years after completing IFN-α therapy. The patient was a non-responder, female with HBV infection and with increased baseline and follow-up AFP levels. On the basis of these results we recommend hepatocellular cancer screening at short intervals for every patient treated with IFN-α after therapy. The fact that there was no HCC development in patients with a complete and sustained response to IFN-α treatment provides

hope for this therapy in chronic viral hepatitis. Further studies are required to clarify the role of IFN α in protecting malignant hepatocyte transformation in patients with HBV and HCV-related chronic liver disease.

CONCLUSIONS

1. IFN- α therapy could diminish the risk of liver carcinogenesis in pts with cvh B and C. It significantly decreases the serum AFP level.

2. Its beneficial effect was observed both in responders and in non-responders.

REFERENCES

1. Alberti A, Pontisso P.: Hepatitis viruses as aetiological agents of hepatocellular carcinoma. Ital. J. Gastroenterol., 23, 7, 452, 1991.
2. Andreone P. et al.: Interferon and hepatocellular carcinoma. Lancet, 347, 8995, 195, 1996.
3. Baffis V. et al.: Use of interferon for prevention of hepatocellular carcinoma in cirrhotic patients with hepatitis B or hepatitis C virus infection. Ann. Intern. Med., 131, 9,696, 1999.
4. Bei R. et al.: Cryptic epitopes on alpha-fetoprotein induce spontaneous immune responses in hepatocellular carcinoma, liver cirrhosis, and chronic hepatitis patients. Cancer Res., 59, 21, 5471, 1999.
5. Cedrone A. et al.: Utility of alpha-fetoprotein (AFP) in the screening of patients with virus-related chronic liver disease: does different viral etiology influence AFP levels in HCC? A study in 350 western patients. Hepatogastroenterology, 47, 36, 1654, 2000.
6. Chu C.W. et al.: Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. J. Clin. Gastroenterol., 32, 3, 240, 2001.
7. Dusheiko G.M.: Hepatocellular carcinoma associated with chronic viral hepatitis. Aetiology, diagnosis and treatment. Br. Med. Bull., 46, 2, 492, 1990.
8. Goldstein N.S. et al.: Serum alpha-fetoprotein levels in patients with chronic hepatitis C. Relationships with serum alanine aminotransferase values, histologic activity index, and hepatocyte MIB-1 scores. Am. J. Clin. Pathol., 111, 6, 811, 1999.
9. Gramenzi A. et al.: Impact of interferon therapy on the natural history of hepatitis C virus related cirrhosis. Gut, 48, 6, 843 2001.
10. Hino O., Kajino K.: Hepatitis virus-related hepatocarcinogenesis. Intervirology, 37, 2, 133, 1994.
11. Hoofnagle J. H., Di Bisceglie A. M.: The treatment of chronic viral hepatitis N. Eng. J. Med., 336, 5, 347, 1997.
12. Mazzella G. et al.: Alpha interferon treatment may prevent hepatocellular carcinoma in HCV- related liver cirrhosis. J. Hepatol., 24, 2, 141, 1996.
13. Schafer D.F, Sorrell M.F.: Hepatocellular carcinoma. Lancet, 353 (9160), 1253, 1999.
14. Sherman M.: Hepatocellular carcinoma. Gastroenterologist, 3, 1, 55, 1995.
15. Shindo M. et al.: Varying incidence of cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis C responding differently to interferon therapy. Cancer, 85, 9, 1943, 1999.

SUMMARY

The aim of our study was assessment of the long-term influence of interferon-α (IFN-α) treatment on the serum marker of the hepatocarcinogenesis level–α–fetoprotein (AFP)-in patients (pts) with chronic viral hepatitis (cvh) B and C. Thirty seven pts (21 with HCV and 16 with HBV infection (20 women, 17 men, aged 24–62) were included in the study. Pts were administered IFN–α in the dose of 9–15 MU per week, thrice a week, for 16 weeks (HBV group) or 24–52 weeks (HCV group). Effectiveness of IFN-α treatment was evaluated on the basis of the HBV DNA and HCV RNA level in blood. The serum AFP values were determined before and 4–7 years after IFN- α treatment.The baseline serum AFP level was increased in 26 out of 37 pts (70%) (14/21 from HCV group; 12/16 from HBV group). After the 4–7 years' follow-up it remained increased only in 2 out of 37 pts (5%). AFP values significantly decreased after IFN-α treatment (17.58 ± 19.09 IU/ml vs 7.95 ± 21.78 IU/ml; $p < 0.05$; normal range 0–5 IU/ml) in both HBV and HCV, responder and non- responder groups. These results support the hypothesis that IFN-α therapy could diminish the risk of liver carcinogenesis in pts with cvh B and C. It significantly decreases the serum AFP level. Its beneficial effect was observed both in responders and in non- responders.

Terapia interferonem-α może zmniejszać występowanie raka wątroby u pacjentów z przewlekłymi wirusowymi zapaleniami wątroby

Celem badania była ocena długoterminowego wpływu leczenia interferonem–α (IFN–α) na poziom osoczowego markera karcynogenezy– α–fetoproteiny (AFP) u pacjentów z przewlekłym wirusowym zapaleniem wątroby typu B i C (pwzw B i C). Do badania zakwalifikowano 37 chorych (21 z infekcją HCV i 16 z infekcją HBV; 21 kobiet; 17 mężczyzn, w wieku 24–62 lata). Pacjentom podawano IFN–α w dawce 9–15 MU tygodniowo, trzy razy w tygodniu, przez 16 tygodni (grupa HBV) lub 24–52 tygodni (grupa HCV). Skuteczność leczenia IFN–α oceniano na podstawie obecności HBV DNA lub HCV RNA w surowicy krwi. Poziom AFP w osoczu badano przed oraz 4–7 lat po zakończeniu leczenia IFN–α. Podstawowy poziom AFP w surowicy krwi był podwyższony u 26 z 37 chorych 70% (14/21 w grupie HCV, 12/16 w grupie HBV). Po 4–7 latach obserwacji poziom AFP pozostał podwyższony tylko u 2 z 37 pacjentów (5%). Wartości AFP po leczeniu IFN–α obniżyły się istotnie w obu grupach chorych (HBV i HCV), zarówno u osób z dobrą odpowiedzią, jak i bez odpowiedzi na leczenie (przed IFN–α 17,58 ± 19,09 IU/ml vs, po IFN–α 7,95 ± 21,78 IU/ml; $p < 0,05$; normal range 0–5 IU/ml). Uzyskane wyniki wskazują na to, że terapia IFN–α może zmniejszać ryzyko wystąpienia raka wątroby u pacjentów z pwzw B i C. Istotnie obniża ona poziom AFP w osoczu. Ten korzystny efekt był obserwowany w grupie chorych z dobrą odpowiedzią i u chorych bez odpowiedzi na leczenie IFN–α.