Original article

Co-administration of granulocyte colony-stimulation factor allows completion of interferon therapy in chronic viral hepatitis with neutropenia

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SUMMARY

Neutropenia, due either to hypersplenism or to myelotoxicity of interferon (IFN), is a contraindication to start or continue IFN treatment. In a prospective, open study the use of granulocyte colony stimulating factor (G-CSF), a growth factor capable of directly and selectively stimulating proliferation, differentiation and function of neutrophils, has been evaluated in combination with IFN A-2a in patients with chronic viral hepatitis. Inclusion criteria, in addition to those for IFN treatment, were compensated disease (Child-γ class A), leukocyte count< 3,0 k/μl and/or neutrophil count < 1,5 k/μl. The dose of G-CSF was titrated in order to raise and maintain the white cell counts above these levels throughout the treatment period and limited to <5μg/Kg subcutaneously daily. Twelve patients (F: 7, M: 5, mean age 45,7, range 23-59), 7 with B and 5 with C infection entered the study. Ten of these, 5 with active cirrhosis and 5 with chronic active hepatitis completed a combined interferon + G-CSF therapy period of 6 months, with a mean total G-CSF dose of 10,5 mg (range 4,8-21.6 mg) per patient. In the other two patients, treatment was stopped at 3 and 4 months because they developed ascites and peripheral oedema due either to loss of compensation or to fluid retention caused by G-CSF. No other important side-effects were observed and, in particular, no excess leucocytosis. At the end of the treatment period, 4 patients showed complete biochemical and virological response to IFN, 4 partial and another 2 no response. These preliminary results suggest that prolonged administration of G-CSF is relatively safe in chronic viral liver disease and allows completion of a course of IFN therapy in neutropenic patients.

Key words: G-CSF, Granulocyte colony- stimulating factor, Hepatitis, Therapy, Interferon, Neutropenia

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INTRODUCTION

It is known that infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV) leads to chronicity in approximately 10% and 70% of cases respectively. A significant proportion of these patients with active hepatitis will develop cirrhosis. On the other hand there is a strong relationship between cirrhosis due to HBV or HCV and hepatocellular carcinoma (HCC). Both diseases have an increased mortality rate[1-3].

Interferon-a (IFN) has been relatively effective in the treatment of chronic hepatitis B or C, leading to inhibition of active viral replication and sometimes remission of hepatic inflammatory changes in a percentage of cases[4-7].

However, the use of this antiviral agent has been associated with various side effects. Myelotoxicity is one of them, which results in neutropenia and often leads to the cessation of treatment. On the other hand, neutropenia as a result of hypersplenism of chronic liver disease is a contraindication to start or continue IFN treatment. Hence neutropenic patients with chronic viral hepatitis are deprived of the possible benefits of this treatment.

Granulocyte colony-stimulating factor (G-CSF) is a naturally occurring glycoprotein, which directly and se-

lectively stimulates proliferation, differentiation and functional activity of neutrophils[8,9]. G-CSF has been approved by the FDA for use in patients with non-myeloid malignancies on cytotoxic drugs[10,11] and has been administered successfully to cirrhotic patients with hypersplenism in order to increase the peripheral neutrophil count, as well as to liver allograft recipients with viral hepatitis[12,13].

Our pilot study was designed to evaluate the efficacy of G-CSF in combination with IFN in patients with chronic hepatitis B or C and neutropenia and in particular, whether or not it allows completion of IFN therapy without significant side-effects.

PATIENTS-METHODS

A total number of 12 patients (Males=5, Females=7; mean age=45,7, range=23-59 years) were enrolled in the study. Seven patients had chronic hepatitis B and 5 had chronic hepatitis C.

Entry criteria included: 1) Age between 18-70 years. 2) Presence of the viral markers HsBAg and serum HBV-DNA for the patients with chronic hepatitis B and anti-HCV antibodies and serum HCV-RNA for the patients with chronic hepatitis C. 3) Increased serum alanine aminotransferase (ALT) level at least 1,5 times the upper limit of normal (4-40u/L). 4) Compensated liver disease (prothrombin time prolongation <3 sec, serum albumin level >3mg/dl, bilirubin level <3mg/dl, no history of hepatic encephalopathy, bleeding esophageal varices or ascites). 5) Leucocyte counts <3,0 k/μl and/or neutrophil count<1,5-k/μl before treatment. 6) Exclusion of any other liver disease.

Liver biopsy, performed within 6 months prior to study entry revealed 6 patients with chronic active hepatitis and 6 patients with active cirrhosis.

All patients received recombinant interferon alpha-2a (Roferon-A, Hoffman La Roche) at doses of 3-9 MU, 3 times a week, subcutaneously, for a period of 6 months.

On entry to the trial the G-CSF (Granulokine, Hoffman La Rhoce) was titrated for each patient during a 7 day hospitalization, in order to maintain the white cell counts>3,0 k/μl and the neutrophil counts>1,5k/μl throughout the treatment period. It was given subcutaneously, 1-3 times weekly and ranged between 2,5 and 5 μg/ Kg body weight.

During therapy the patients were followed up by clinical examination and laboratory analysis (including hematological and biochemical test) daily during the first week of therapy and fortnightly thereafter on an outpatient basis. We analyzed data with the paired t-test.

All the patients gave their written consent to participate in the trial.

RESULTS

Ten patients completed a combined IFN+G-CSF therapy period of 6 months. The mean total G-CSF dose was 10,5 mg (range: 4,8-21,6 mg) per patient. The pre-

Table. Pretreatment and end-treatment characteristics of patients who completed the combined treatment.

							Before Rx			End-of Rx		
Pts	**Age**	**Sex**	**Chronic**	**Histological**	**G-CSF**	**IFN dose**	**WBC**	**NBC**	**ALT**	**WBC**	**NBC**	**ALT**
(no)	**(year)**		**hepatitis**	**diagnosis**	**dose**	**(MU/tiw)**	**(k/μl)**	**(k/μl)**	**(u/l)**	**(k/μl)**	**(k/μl)**	**(u/l)**
1	58	M	HCV	CI	300 μg/qwx6mo.	6	2.8	1.2	161	17.9	14.6	127
2	54	F	HBV	CI	300 μg/biwx6mo.	5	2.0	1.4	135	16.1	13.7	44
3	27	M	HBV	CAH	600 μg/tiwx3mo.	9	1.8	0.9	47	6.8	5.8	87
4	27	F	HCV	CAH	300 μg/qwx6mo.	3	2.9	1.4	469	8.0	6.7	217
5	52	F	HCV	CI	150 μg/biwx6mo.	3	2.9	1.2	56	5.4	3.0	32
6	44	F	HBV	CAH	300 μg/qwx6mo.	4.5	2.2	0.9	309	3.5	2.6	100
7	59	F	HCV	CI	300 μg/biwx6mo.	3	2.1	0.8	87	4.4	3.8	40
8	52	F	HCV	CI	300 μg/biwx6mo.	3	1.6	1.3	82	7.2	6.2	19
9	53	M	HBV	CAH	150 μg/biwx6mo.	4.5	2.3	0.8	153	13.2	10.5	60
10	23	M	HBV	CAH	150 μg/biwx4mo.	9	2.9	1.4	82	15.5	13.3	55
						M	2.35	1.13	158	9.8	8.02	78.1
						SD	0.41	0.25	133	5.34	4.6	59

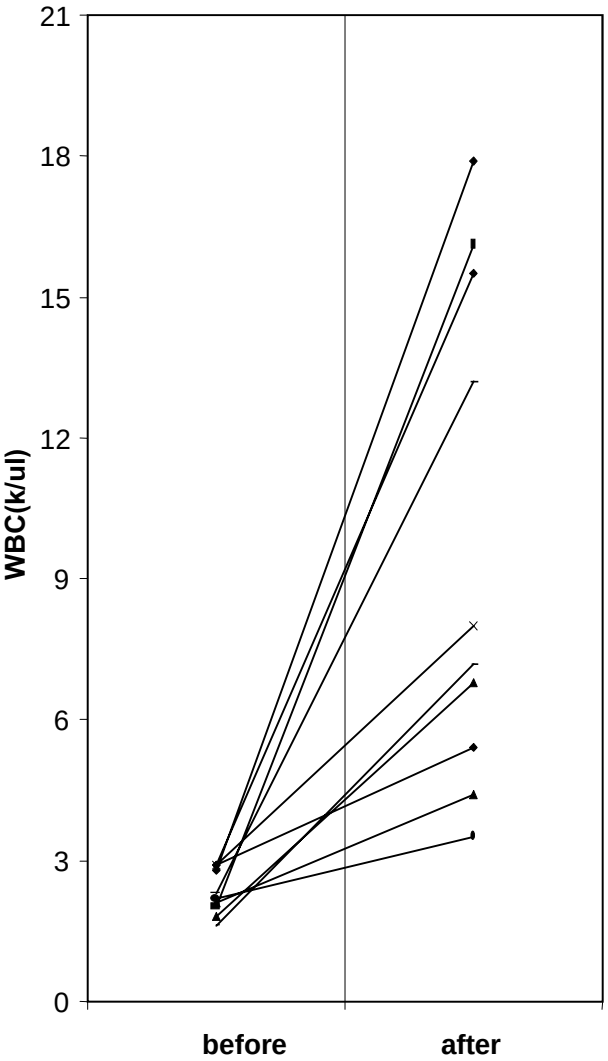

Figure 1. WBC before and after Rx.

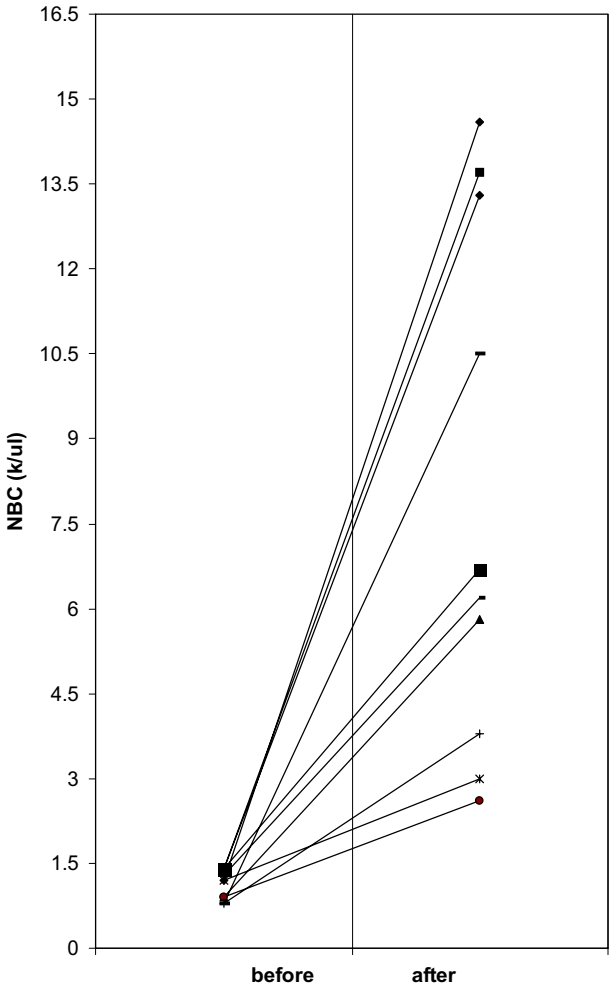

Figure 2. NBC before and after Rx.

treatment and end-of treatment patient characteristics are shown in the table.

There was a significant rise of the mean leukocyte blood cells (WBC) at the end-of treatment (9,8+5,34) compared to that before (2,35+0,41) as shown in Figure 1, (p=0,001).

Likewise, there was a significant rise of the mean neutrocyte blood cells (NBC) at the end-of treatment (8,02+4,6) compared to that before (1,13+0,25) as shown in Figure 2, (p<0,001).

Regarding the response to IFN treatment, 4 patients showed complete biochemical and virological response, 4 partial and another 2 no response. These two patients were not analyzed further.

Two patients dropped out because of the development of ascites and peripheral oedema after 3 and 4 months of treatment respectively.

The safety at G-CSF was satisfactory in all the other patients. No other side-effects were reported or observed and in particular no excessive leucocytosis or the presence of immature granulocytosis.

DISCUSSION

In this trial, we administered G-CSF in combination with IFN to patients with chronic hepatitis B or C and neutropenia in order to study the potential benefit of this combination in allowing the completion of an IFN course.

The two basic phagocytic cells, neutrophils and macrophages, develop from a common progenitor cell, called

the granulocyte/macrophage (or GM) progenitor cell. Like other granulocytes (eosinophils and basophils), neutrophils circulate in the blood for only a few hours before migrating out of capillaries into the connective tissue or other specific sites, where they survive for only a few days dying and being phagocytosed by IFN. By contrast, macrophages can persist for months or perhaps even years outside the bloodstream, where they can be activated by local signals to resume proliferation[14].

Neutrophils, are the most numerous circulating white blood cells and the principal elements of the anti-infective defense. Neutropenia is defined as a circulating peripheral neutrophil count of less than $2,0X10^9$/L.

At least seven distinct Colony-stimulating factors (CSFs), which stimulate neutrophil and macrophage colony formation in culture, have been defined, and some or all of these are thought to act in different combinations to regulate the selective production of these cells in vivo. These CSFs are synthesized by various cell types - including endothelial cells, fibroblasts, macrophages, and lymphocytes - and their concentration in the blood typically increases rapidly in response to bacterial infection in a tissue, thereby increasing the number of phagocytic cells released from the bone marrow into the bloodstream. All of these CSFs, are glycoproteins that act at low concentrations (´10-12 M) by binding to specific cell-surface receptors. They are now being used in patients to stimulate the regeneration of hemopoietic tissue and to boost resistance to infection[14].

The G-CSF is a glycoprotein, which belongs to a large receptor family, sometimes called the cytokine receptor family[15]. This factor enhances the production of the neutrophils in bone marrow and spleen, induces an increase in circulating neutrophils. It also affects their functional properties and controls the activation, proliferation and maturation of these cells.

According to our study results, the use of G-CSF was generally well tolerated and led to significant increases in the numbers of WBCs in all patients, who had leucocyte count <3,0k/μl and, or neutrophil count <1,5m/μl before the start of IFN treatment. These results were expected given the properties of the G-CSF mentioned above. No excessive leucocytes were observed during treatment because the dose titration was meticulous and in any case such side effect has not been reported with therapeutic doses of the drug in humans. In addition, no excessive numbers of immature leucocytes were observed. It should be also noted that the safety profile of the drug was satisfactory and only two patients were withdrawn from the study during therapy due to development of ascites and peripheral oedema. It is not known whether these events were due to fluid retention, which is a reported side-effect of G-CSF administration, or to loss of compensation of cirrhosis, which is the most probable.

The possible role of G-CSF as a drug that could have a synergistic effect with IFN in chronic hepatitis should be evaluated. G-CSF specific cell-receptors have been observed in various cells but not in lymphocytes, which are the principal cells of cell-mediated immune response in chronic viral hepatitis[16]. Likewise, there have been in vitro studies demonstrating that certain cytokines involved in inflammation can induce bone marrow and other normal cells to produce G-CSF. On the other hand, it was shown in a simple model that the maintenance of B-cell potential of the primitive progenitor cells appears to require interactions of early acting cytokines including IL-6, G-CSF, IL-11, IL-12, LIF, and SF and this fits with our understanding of the interactions of growth factors with hematopoietic progenitors[17]. However, there have not been enough data to support a significant role for G-CSF in the response to infection. So, on a theoretical basis, we don't know if G-CSF has a synergistic effect with IFN in the treatment of chronic hepatitis. Only 4 of 10 patients who completed the 6 month combination therapy with IFN alpha-2a and G-CSF showed a complete end-of-therapy response. This does not support a synergistic role for G-CSF with IFN in the treatment in chronic hepatitis. However, it would be interesting to further investigate this possibility.

In our study the use of G-CSF in patients with chronic hepatitis B and C, who otherwise could not have been treated with IFN due to neutropenia, allowed the completion a 6 month course of treatment in most treated patients with reversion of neutropenia and prevention of possible bacterial infections during IFN treatment.

In conclusion, the administration of G-CSF in combination with IFN in patients with chronic hepatitis B and C allows completion of IFN therapy without significant side-effects. These preliminary results suggest that prolonged administration of G-CSF is relatively safe and could have a place in the treatment of selected neutropenic patients with chronic hepatitis. This is particularly true for patients with chronic hepatitis C for whom there is no alternative drug to IFN at present, in contrast to chronic hepatitis or cirrhosis B patients with neutropenia, who can now be treated with nucleocide analogues such as lamivudine.

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