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Int Hepatol Commun, 2 (1994) 161–165

International
Hepatology Communications

Elevated serum levels of macrophage colony stimulating factor during interferon-α therapy for chronic hepatitis C

Yoshito Itoh, Takeshi Okanoue*, Fumio Enjyo, Schinichi Sakamoto, Keizo Kagawa, Kei Kashima

Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyou-ku, Kyoto, Japan

(Received 20 September 1993; accepted 3 December 1993)

Abstract

We estimated the serum levels of macrophage colony stimulating factor (M-CSF) in patients with chronic hepatitis C who were treated with interferon-α (IFNα). In 12 patients who received 3 or 6 MU of natural IFNα, or 9 MU of recombinant IFNα daily for 2 weeks then three times a week, blood was collected every 24 h after injection of IFNα in the initial 7 days of the therapy. In all the patients, serum levels of M-CSF were significantly ($P < 0.05$) higher 24 h after the first injection of INFα. After reaching plateau levels on the third day, M-CSF remained at high levels. In four patients who received 3 or 6 MU of natural IFNα daily for at least 2 weeks, blood was collected daily three times after the last injection of IFNα. In all four patients, serum M-CSF levels gradually fell to the pre-treatment levels after 72 h. In six patients who received 3 or 6 MU of natural IFNα for 8 weeks, blood was collected before, during, and after the therapy. In these patients, serum M-CSF levels continued to be high during the therapy and decreased to pre-treatment levels after finishing the therapy. These results indicate that IFNα administration would increase serum levels of M-CSF in vivo.

Key words: M-CSF; IFNα; Hepatitis C

1. Introduction

It is well known that IFNα possesses multiple functions, such as anti-tumor, anti-viral, and immune regulatory functions [1]. Recently, IFNα has been widely used for the treatment of chronic hepatitis B and C [2,3]. Clinical occurrence of immunological abnormalities such as autoimmune thyroid disease [4], autoimmune hepatitis [5], or abnormal production of interleukin-6 [6] has been reported in the minority of patients receiving IFNα. The previous publications reported that IFNα administration de-

*Corresponding author.

SSDI 0928-4346(93)E0072-X

creased serum thyroid hormone levels [7] and increased cortisol levels or triglyceride levels [7,8], whereas little is known about the effects of IFNα on the levels of hematopoietic factors such as M-CSF in vivo. In the present preliminary report, we show that levels of M-CSF in sera may be upregulated by the administration of IFNα for chronic hepatitis C patients.

2. Materials and methods

2.1. Patients

Twenty-two patients with chronic hepatitis C were studied. Informed consent was obtained from all the patients before IFNα therapy, and this study was approved by the ethical committee of the hospital. The diagnosis of chronic hepatitis C was based on a positive 2nd-generation ELISA (Ortho Diagnostic System, Raritan, NJ, USA) and liver biopsy.

2.2. IFNα administration

Twenty-two patients were divided into three groups. In twelve patients who received either 3 MU or 6 MU of natural IFNα (Sumitomo Pharm. Co., Osaka, Japan) or 9 MU of recombinant IFNα-2a (Takeda Pharm. Co., Osaka, Japan) for 2 weeks then three times a week, blood was collected before and every 24 h in the initial 7 days. In four patients who received 3 or 6 MU of natural IFNα for at least 2 weeks, blood was collected three times every 24 h after the last injection of IFNα. In six patients who received IFNα daily for 8 weeks, blood was collected before, at the end of the 1st, 2nd, 4th, and 8th week, then 2 weeks after finishing the therapy. Samples were stored at −20°C for the assay.

2.3. Radioimmunoassay of M-CSF

The radioimmunoassay for M-CSF was prepared by the method we previously reported [9]. In brief, duplicate sera and the standard recombinant human M-CSF (rhM-CSF; 100 μl) were mixed with a I^{125}-labeled rhM-CSF (10000 cpm/100 μl) and rabbit anti-rhM-CSF suspension (200 μl). After incubating for 48 h at 37°C, the binding product was separated from free I^{125}-labeled rhM-CSF by the addition of anti-rabbit IgG and 6% polyethylene glycol. The tubes were centrifuged and the supernatants were aspirated. The precipitates were counted for 1 min in a gamma spectrometer. The sensitivity limit was 0.1 ng/ml in this assay. Data are expressed as the mean value ± standard deviation. Paired *t*-test was used to evaluate the statistical significance, which was determined with a *P*-value under 0.05.

3. Results

As shown in Fig. 1A, serum levels of M-CSF were significantly high 24 h after the first administration of IFNα ($P < 0.05$). The mean serum levels of M-CSF increased on the third day, reaching to the plateau levels. Then, the mean levels of M-CSF

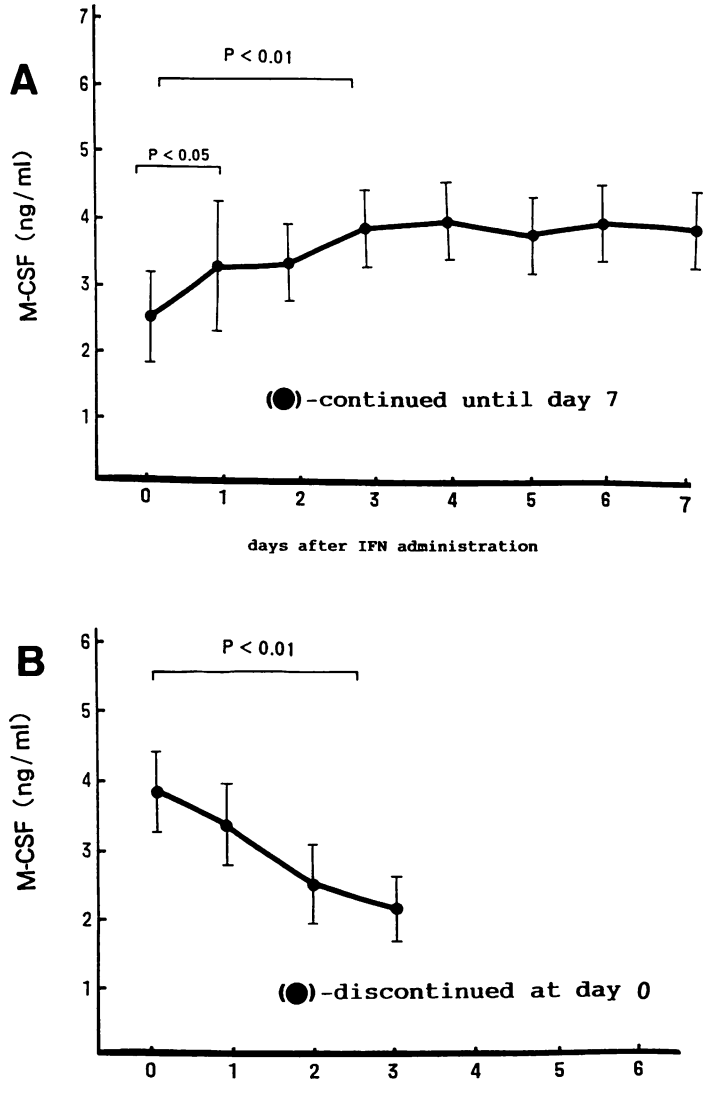

Fig. 1. (A) In a patient who received IFNα daily, serum M-CSF levels were significantly high 24 h after the first administration of IFNα. After reaching the plateau level on the third day, levels of M-CSF remained in high levels until the 7th day. (B) In a patient who ceased to receive IFNα, serum M-CSF levels decreased to pre-treatment levels (2.32 + 0.21 ng/ml) after 72 h.

continued to be high until the 7th day. However, in all the patients who ceased to receive IFNα (Fig. 1B), M-CSF decreased to low levels comparable to the pre-treatment ones (2.32 ± 0.21) after 72 h. In this study, three kinds of protocols (3 MU of natural IFNα, 6 MU of natural IFNα, and 9 MU of recombinant IFNα) were used. No significant differences were noted among these three protocols.

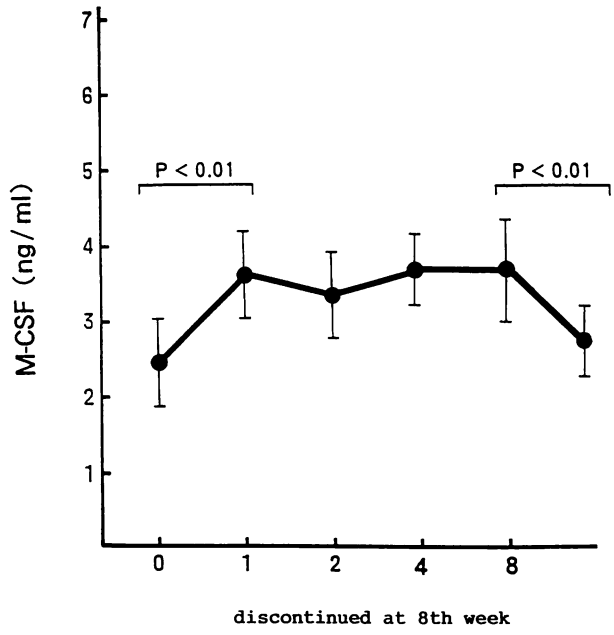

Fig. 2. In a patient who received IFNα daily for 8 weeks, serum M-CSF levels increased and remained in high levels during the therapy. However, after finishing the IFNα therapy, serum levels of M-CSF decreased to pre-treatment ones.

As shown in Fig. 2, serum levels of M-CSF were determined in patients with chronic hepatitis C who received IFNα daily for 8 weeks. The mean serum M-CSF levels were significantly high in the first week and continued to be high until the end of the protocol. However, serum M-CSF levels decreased to pre-treatment levels 2 weeks after finishing IFNα therapy. No significant differences were noted in M-CSF levels among the patients who received different amounts of IFNα.

4. Discussion

According to a recent study on IFN therapy for chronic hepatitis C, the anti-viral effect of IFNα is enhanced in proportion to the dosage and duration of IFNα [10]. A variety of side effects induced by IFN therapy have been reported [3–5]. Supposing that IFNα will be used in higher doses and for longer periods in the future, the effects of IFNα should be monitored not only from an immunological aspect, but also from endocrinological, neurological and hematological aspects.

In this paper, we showed, for the first time, that serum M-CSF levels increase in chronic hepatitis C patients treated with IFNα. Serum M-CSF levels were higher 24 h after the first administration of IFNα. Patients who received IFNα for 8 weeks

continuously show high levels of M-CSF in sera. After discontinuation of IFNα, serum M-CSF levels decreased after 72 h.

Although serum levels of M-CSF increased during the IFN therapy, no significant increase in the monocyte counts was observed. Because a substantial amount of M-CSF is consistently present in the sera of control patients [9,11], pathological significance of elevated serum M-CSF levels during the IFNα therapy might be questionable. Further studies will clarify this issue.

It has been reported that M-CSF is produced by various types of cells including monocytes, T cells, bone marrow stroma cells, and fibroblasts [11,12]. Therefore, it remains to be clarified which cells are involved in the upregulation of serum M-CSF levels during IFNα therapy in vivo in the future.

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