NOTE

Increase in Serum Interleukin-6, Plasma ACTH and Serum Cortisol Levels After Systemic Interferon-α Administration

HIROYUKI SHIMIZU, KEN-ICHI OHTANI, NORIYUKI SATO, TAKEAKI NAGAMINE, AND MASATOMO MORI

First Department of Internal Medicine, Gunma University School of Medicine, Gunma 371, Japan

Abstract. Systemic administration of human interferon-α stimulates the pituitary-adrenal axis in men, but the exact mechanism still remains to be established. The present study was undertaken to examine the hypothesis that interferon-α may alter the circulating concentrations of the cytokines which involve the activation of the pituitary-adrenal axis. Eleven patients with chronically active hepatitis C were treated with human lymphoblastoid interferon-α (IFN: 6 x 10⁶ IU/day) and changes in plasma adrenocorticotropin (ACTH), serum cortisol and cytokine concentrations were observed on both the first and second days of the treatment. Subcutaneous administration of IFN significantly increased plasma ACTH and serum cortisol concentrations by 3 h after the injection. Serum interleukin-6 (IL-6) increased with the increase in circulating ACTH and cortisol. There was a significant correlation between serum cortisol and IL-6 concentrations at 3 h. In contrast, an increase in serum interleukin-1β was only observed in one case. On the second day of IFN treatment, simultaneous administration of 25 mg diclofenac sodium eliminated the IFN effects on circulating ACTH, cortisol and IL-6 concentrations. The present studies demonstrated that circulating IL-6 increases after systemic IFN administration, resulting in activation of the pituitary-adrenal axis.

Key words: Interleukin-6, ACTH, Cortisol, Interferon-α, Chronic hepatitis

(RECENTLY, the therapeutic efficiency of interferon-α has been shown for chronically active hepatitis C [1]. Interferon-α is now available for the treatment of active viral hepatitis, but there appear to be lots of problems in systemic interferon administration. Systemic administration of interferon-α increases body temperature, induces anorexia at the beginning of the treatment, and often suppresses hematopoiesis in the bone marrow [2, 3]. From the endocrinological aspect, it is well known that various cytokines, released from invaded inflammatory cells such as macrophages, affect the regulation of neuroendocrine function following peripheral immune reaction. Recently, Muller et al. reported that interferon-α-2 stimulates ACTH and cortisol secretion in men [4]. While interferon-α can act as a mediator between the immune and endocrine systems, it does not appear to enter the brain since it is a polypeptide unable to penetrate the blood brain barrier [5]. Smith and his colleagues also demonstrated that the immunoactivity of human interferon-α was not detected in cerebrospinal fluid after intravenous administration of 18 x 10⁶ IU interferon-α [6]. Furthermore, McGillis and his associates could not find ACTH release from cultured pituitary cells after direct stimulation by interferon-α [7]. Therefore, the ex-
act mechanism by which systemically administered interferon-α stimulates ACTH and cortisol secretion in man still remains to be established.

Interleukin-1β and interleukin-6 have been reported to activate the pituitary-adrenal axis [8, 9]. Progress in medical technology enabled us to measure the circulating concentrations of various cytokines. The present studies were undertaken to determine the effect of interferon-α on the circulating concentrations of these cytokines.

**Subjects and Methods**

**Subjects**

The studies included 11 patients (4 male and 7 female) who suffered from chronically active hepatitis C without any endocrine disorders. These patients were in a clinically stable state without signs of hepatic failure. The average age was 54.2 ± 2.2 years. Chronically active hepatitis C was diagnosed by histological observation of specimens obtained by needle biopsy. Before the study, the aim of the examination was explained and informed consent was obtained from all patients included in the present study.

**Experimental design**

The patients were treated with $6 \times 10^6$ IU human lymphoblastoid interferon-α (IFN: Sumitomo Pharmaceuticals, Tokyo, Japan) every day for at least 2 weeks [10]. IFN, dissolved in saline, was subcutaneously injected every morning at 0900 h. As mentioned below, changes in humoral factors were observed for 3 h on the first 2 days of the treatment.

On the first day of the treatment, blood samples were drawn from the ante-cubital vein before and 3 h after subcutaneous IFN injection. Our preliminary study suggested that plasma ACTH and serum cortisol concentrations were increasing from 2 to 3 h after IFN administration in parallel with the increase in circulating interleukin-6 when body temperature began to rise (Fig. 1). To avoid the effect of body temperature rise, we selected this period (3 h). From the samples collected, plasma and serum were separated by centrifugation at low speed and then stored at −20 °C until radioimmunoassay.

On the second day of the treatment, 25 mg of diclofenac sodium [11] was administered before IFN administration and blood samples were collected as on the first day of treatment.

**Assay**

Plasma adrenocorticotropic (ACTH), serum cortisol, interleukin-1β (IL-1) and interleukin-6 (IL-6) concentrations were determined with radioimmunoassay kits obtained from Japan Mediphysics Co., Baxter Co., Medgenix Co. and Fuji Rebio Co. (Tokyo, Japan), respectively.

**Statistics**

Data were expressed as the mean ± SEM. Statistical analysis of the data was performed by analysis of variance, followed by paired $t$-test for individual comparison of the means.
Results

Figures 2 and 3 show the changes in circulating ACTH, cortisol, IL-1 and IL-6 concentrations before and 3 h after the first IFN injection in all eleven patients. As shown in Fig. 2, IFN administration significantly increased plasma ACTH and serum cortisol at 3 h after IFN injection (ACTH: before, 17.5 ± 2.6 pg/ml; at 3 h after, 151.6 ± 53.5 pg/ml, P<0.05, Cortisol: before, 10.91 ± 0.74 μg/dl, at 3 h after, 19.96 ± 1.65 μg/dl, P<0.01). Neither IL-6 (<4.0 pg/ml) nor IL-1 (<10 pg/ml) were detectable in the serum before IFN administration. Serum IL-6 significantly increased by 3 h after IFN administration (Fig. 3), but an increase in serum IL-1 was only observed in one patient.

Figure 4 demonstrated the correlation between serum cortisol and IL-6 concentrations at 3 h after the first IFN administration. There was a significantly positive correlation between these factors (r=0.685, P<0.05), but there was no significant correlation between plasma ACTH and serum IL-6 concentrations on the same period (r=0.223, P>0.05).

Figure 5 demonstrated the effects of 25 mg diclofenac sodium administration on the increase in plasma ACTH, serum cortisol and IL-6. Simultaneous administration of diclofenac sodium with IFN inhibited an increase in body temperature after IFN administration. In the presence of diclofenac sodium, increases in plasma ACTH and cortisol concentrations were significantly lower than those in the IFN only group.

Fig. 2. Changes in plasma ACTH and serum cortisol concentrations before and at 3 h after the first injection of human lymphoblastoid interferon-α (6 x 10^6 IU/day). Open circles and bars show the mean ± SEM.

Fig. 3. Changes in serum interleukin-1β (IL-1) and IL-6 concentrations before and at 3 h after the first injection of human lymphoblastoid interferon-α (6 x 10^6 IU/day). Open circles and bars show the mean ± SEM. n.s.: statistically not significant.

Fig. 4. Correlation between serum cortisol and interleukin-6 (IL-6) concentrations at 3 h after human lymphoblastoid interferon-α (IFN: 6 x 10^6 IU/day) administration.
serum cortisol were obviously attenuated. Similarly, an increase in serum IL-6 was significantly reduced by simultaneous administration of diclofenac sodium.

**Discussion**

The present studies demonstrated that systemic IFN administration increased circulating ACTH, cortisol and IL-6 in the patients with chronically active hepatitis C. There was a significantly positive correlation between serum cortisol and IL-6 concentrations at 3 h after IFN administration (Fig. 4). Recently, a case report by Itoh and his colleagues first suggested that $6 \times 10^6$ units of recombinant α2a-interferon increased serum IL-6 in a patient with hepatitis B [12]. The results of the present study are compatible with their finding and also showed that serum cortisol and IL-6 concentrations were correlated 3 h after IFN administration.

The present study demonstrated that plasma ACTH and serum cortisol increased with the increase in circulating IL-6 ($12.7 \pm 2.1$ pg/ml). When the H-P-A axis was activated by endotoxin, the circulating IL-6 concentrations increased from 1 pg/ml (basal) to 91 pg/ml (maximal) [13]. Navarra et al. [14] demonstrated that IL-6 at concentrations of $10^{-13}$ M (about 3 pg/ml) stimulated the in vitro release of ACTH after a 2-h incubation, compared with non-IL-6 added controls. Since the observed increase in circulating IL-6 was thought to be great enough to stimulate ACTH secretion, an increase in circulating IL-6 is supposed to involve the activation of pituitary-adrenal axis by systemic IFN administration.

In addition, we examined the correlation between IL-6 and ACTH, cortisol concentrations. Circulating IL-6 levels and serum cortisol concentrations were significantly correlated, but not plasma ACTH. One of the reasons may be that there is a difference among the patients in the responsibility of pituitary ACTH secretion for increased serum IL-6 concentrations and adrenal cortisol secretion for increased plasma ACTH levels. On the other hand, chronically administered IL-6 (30 μg/kg) may itself stimulate adrenal cortisol secretion in concert or in synergy with pituitary ACTH [15]. IL-6 has been reported to be expressed in the adrenals [16]. Another possibility is that circulating IL-6 may directly affect cortisol secretion in vivo.

Data on the direct effects of IFN on hypothalamic CRH release are controversial. The addition of IFN (10–1000 U/ml) had no effect on hypothalamic CRH-41 release or pituitary ACTH release in the
in vitro experiment [14]. In contrast, Gisslinger et al. recently demonstrated that the addition of IFN stimulated in vitro CRH secretion, while the concentration of IFN used (>10^{-7} M), which stimulated CRH release, appears to be very high range compared with circulating IFN after subcutaneous IFN administration (from 10^{-10} M to 10^{-9} M) [17]. There remains the possibility that IFN directly affects CRH release in vivo.

Circulating IL-1, which stimulates ACTH secretion via hypothalamic CRH release [18-20], was below the detection limit of 5 \times 10^{-13} M except in one case. The minimum concentration which stimulates CRH-41 release from the hypothalamus is 10^{-10} M [12] and the concentration below the detection limit does not appear to be high enough to activate the H-P-A axis.

IL-6 stimulates the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase-dependent pathway [14]. Diclofenac sodium inhibits eicosanoid synthesis through blocking cyclooxygenase [11]. The present finding that diclofenac sodium blocked the IFN effects on circulating ACTH, cortisol and IL-6 levels raised the possibility that the activation of H-P-A axis by IFN may be mediated by a cyclooxygenase-dependent mechanism.

The present studies demonstrated that circulating IL-6 concentrations increase after systemic IFN administration, activating the H-P-A axis.

References


