岩手医科大学
審査学位論文
（博　士）

JIMA Vol. 66, No. 3 (Aug. 2014) pp. 101-112.

Original

Effects of subcutaneous administration of pegylated interferon alpha-2b on the behavior and the cytokine levels of serum and brain in rats

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(Received on January 23, 2014 & Accepted on January 26, 2014)

Abstract

Pegylated interferon (PEG-IFN) often induces adverse psychiatric effects, including depression, but the mechanisms are unclear. We examined the relationships between the behavioral changes and serum and brain tissue cytokine levels after subcutaneous administration of PEG-IFN- α2b in rats. During the 4-week study period (once a week administration of PEG-IFN- α2b), the locomotor activity (LA) was continuously measured every hour. The daytime LA of the PEG-IFN- α2b group was significantly higher in the second week compared with that of the control group. The daily nighttime LA on the second and the third days of the first week were significantly decreased in the control group, but not in the PEG-IFN- α2b group. The immobility time in the forced swim test (FST) was increased only at the point 24 hours after the PEG-IFN- α2b administration. The levels of several cytokines in the brain tissues were significantly decreased at the point of 24 hours without FST, but there were no difference in the serum level. The cytokine levels in both the serum and brain tissues were decreased after the FST. These results indicate that the LA, immobility time and cytokine levels of rats are influenced in the early stage after subcutaneous administration of PEG-IFN- α2b.

Key words : pegylated interferon, depression, locomotor activity, forced swim test, cytokine

I. Introduction

Interferon alpha (IFN- α), a type I interferon, affects the production of cytokines and induces the immune-mediated clearance of tumors and viruses. IFN- α is widely used for many malignant and viral diseases, including chronic hepatitis C (CHC) virus infection [1]. Because pegylated interferon (PEG-IFN)- α is a recombinant type I interferon and has a long half-life biologically, PEG-IFN- α 2a or 2b is effective when injected once a week, and plays a key role in the current standard antiviral therapy for CHC patients. However, IFN- α and/or PEG-IFN- α often induce adverse side effects, including headache, nausea, fever, eruptions, fatigue, anorexia, insomnia, anxiety and depression. In particular, depression is one of the most serious side effects, and can lead to unpredictable suicide [2] and discontinuation of the therapy. A possible mechanism

responsible for the psychiatric symptoms is that IFN- α disturbs the production and action of cytokines, resulting in effects against the central noradrenergic system, serotonergic systems and hypothalamic-pituitary-adrenal axis[3,4].

Systemic administration of IFN- α disturbs the cytokine cascade in both the periphery and the central nervous system (CNS)[5]. In fact, IFN- α induces or inhibits many cytokines, such as interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-13, granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α) and interferon gamma (IFN- γ) in vitro[6,7]. Peripheral cytokines, including IFN- α , would be able to affect the brain through several possible pathways, such as entering the CNS through areas lacking the blood-brain barrier (BBB), crossing the intact BBB at low rates via putative specific transport mechanisms, inducing adhesion molecules which increase the potential for lymphocytes to cross the BBB, or transmitting signals to the brain through the vagus nerve[3,5]. However, it has not been clarified which pathway(s) is mainly involved. Of note, IFN- α has been shown to exist in the brain tissue of healthy subjects and patients with neurological disorders[8,9]. The IFN- α receptor expressed in the human brain has also been observed on astrocytes and microglia[10]. These cells produce cytokines, which induce the inflammation of the CNS and are implicated in the pathogenesis of neurological disorders. We recently reported that, IFN- α 2b treatment in vitro disturbed the glucose metabolism and reduced the proliferation of human astrocytes[11]. Although we consider that impairment of glucose metabolism and the proliferation of astrocytes would be able to affect other cells in the CNS and disturb behavioral activity, the true mechanism(s) underlying the IFN- α -induced depression are still unclear.

In the present study, we measured the behavioral changes using the spontaneous locomotor activity (LA) and the forced swim test (FST), and evaluated the cytokine levels of serum and brain tissues in normal rats after PEG-IFN- α 2b administration, and evaluated the relationships between them.

II. Materials and Methods

1. Animals

Male Wistar rats weighing 285–370 g at 11 weeks old were obtained from CLEA Japan (Tokyo, Japan). Following an acclimation period in our animal facility, the rats were injected PEG-IFN- α 2b in the studies at 15 weeks old weighing 375-453g. The rats were housed in separate cages with a constant temperature of 23 ± 1°C, and under a 12-h/12-h light-dark cycle (lights on at 7:00 a.m.). Each rat was given a rat diet and tap water ad libitum. The experimental procedures were approved by the animal experimental committee of Iwate Medical University and conformed to the principles of laboratory animal care and the current version of the Japanese law on the protection of animals.

2. Drugs and treatments

PEG-IFN- α 2b (recombinant pegylated interferon alpha-2b; Pegintron, MSD, Tokyo, Japan) was administered subcutaneously. The dosage of PEG-IFN- α 2b was 1.5 μ g/kg, which is the same dosage administered to patients with CHC. In contrast, control rats were administered 0.9% physiological saline (PS).

3. Body weight assessment

The body weights were assessed until the end of the study to evaluate the status of anorexia, which might be induced following the administration of PEG-IFN- α 2b.

4. Locomotor activity of rats in the home cage

The spontaneous locomotor activity (LA) of rats was measured as described previously [12]. The LA counts of each rat in the home cage were automatically recorded every hour for a maximum of 5 weeks using an activity monitoring system (Supermex, Muromachi Kikai, Tokyo, Japan). Data from the Supermex sensor were analyzed and stored on a personal computer with an analytical software program (CompACT AMS software; Muromachi Kikai, Tokyo, Japan). During the first week of the study, we measured the decreasing rates of nighttime LA from the first day to each successive day. The decreasing rate of each day was presented as follow: Δ nighttime LA/ nighttime LA during the first day.

5. Forced swim test (FST)

The immobility during the FST has been reported as a marker representing "depressive-like" behavior in rats treated with IFN [13-15]. In this study, we modified the standard method described by Porsolt et al.[16]. Briefly, the rats were placed individually in a cylinder containing 25 cm of water maintained at 25 ± 1 ℃. The rats were forced to swim for 15 min. After 15 min in the cylinder, they were removed, dried and returned to their individual cages. Twenty-four hours later, the rats were placed in a cylinder again, and the duration of immobility during a 5-minute period was measured. They were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only small movements necessary to keep their heads above water. A time sampling technique was employed whereby the predominant behavior in each 5-sec period of the 5 min test was recorded [17].

6. Serum and brain tissue sampling

At the end of the experiment, the rats were anesthetized by a pentobarbital sodium injection and were sacrificed. After approximately 3 mL of blood was obtained from their hearts, the blood was kept on ice at 4°C and was centrifuged at 13,000g. The serum samples were frozen at − 80 °C until measurement of the cytokine levels. The brain tissue samples from the rats were immediately separated into the frontal, temporal, parietal and occipital cortex, cerebellum, stem, hippocampus and pituitary. Then, they were frozen at − 80 °C until measurement of the cytokine levels.

7. Cytokine analysis

The levels of IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, GM-CSF, IFN- γ and TNF- α were measured in each brain tissue (0.5 mg protein/mL) and serum (0.1 mL) sample using a multiplex fluorescent bead array (Bio-Plex Pro Rat Cytokine Th1/Th2 Panel, 12-Plex; Bio-Rad, MA, USA) validated for rats [18] according to the manufacturer's protocol. The lower limit of detection for each cytokine was as follows: IL-1 α , 0.020 pg/ml; IL-1 β , 0.12 pg/ml; IL-2, 0.020 pg/ml; IL-4, 0.20 pg/ml; IL-5, 0.80 pg/ml; IL-6, 0.20 pg/ml; IL-10, 0.14 pg/ml; IL-12, 0.070 pg/ml; IL-13, 0.010 pg/ml; GM-CSF, 0.013 pg/ml; IFN- γ , 0.18 pg/ml; and TNF- α , 0.010 pg/ml.

8. Experimental design

A flow chart of the experimental design

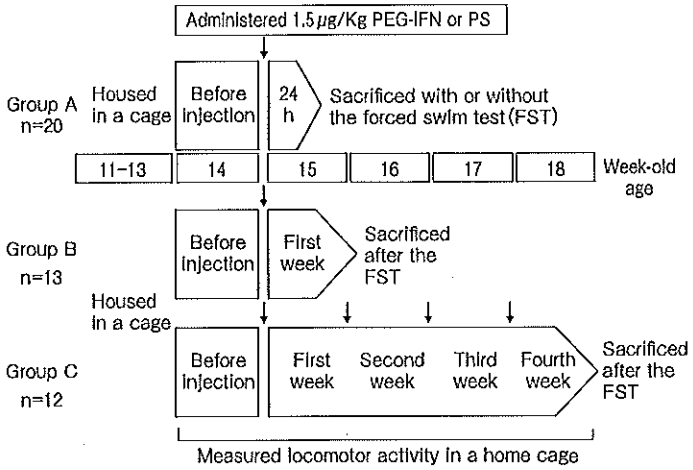

Fig. 1. Experimental design and protocol. At 15 weeks old, male Wister rats on Group A (n=24) were sacrificed after 24 hours from single pegylated interferon (PEG-IFN) α -2b (1.5 μ g/kg) or 0.9% physiological saline (PS) injection (indicated by an arrow). Before sacrifice, 12 of 24 rats were forced swim (PEG-IFN n=6, PS n=6), and the other 12 were not (same above). Group B were measured locomotor activity (LA) for a week from single PEG-IFN or PS injection (n=6, n=7), forced swim and sacrificed. Group C were administered for 4 weeks repeated PEG-IFN or PS injections once a week (n=6, respectively) with LA measuring and forced swim test. Serum and brain tissues were sampled from all groups and measured cytokine levels.

and protocol are shown in Fig. 1. A total of 45 fifteen-week-old rats were used. After the administration of PEG-IFN- α 2b or PS (once a week), the rats were continuously monitored for their LA in their home cages until the end of the study. The FST was performed in both groups of rats (PEG-IFN- α 2b or PS administration) at 24 hours (PEG-IFN- α 2b, 6; PS, 6), 1 week (PEG-IFN- α 2b, 6; PS, 7) and 4 weeks (PEG-IFN- α 2b, 6; PS, 6) after the start of the study, and the rats were then sacrificed. Because the FST is a stressor and might affect the cytokine levels, we also evaluated the cytokine levels in eight rats that did not undergo the FST at the 24-hour time point after a single administration of PEG-IFN- α 2b or PS (PEG-IFN- α 2b, 4; PS, 4).

9. Statistical analysis

All data were expressed as the means ± SEM. The statistical analysis for multiple groups was performed with the Kruskal-Wallis test, followed by the Steel-Dwass test for multiple comparisons. Student's t-test was used for comparisons of the two groups. The Spearman rank coefficient was calculated to assess the correlations between the data. Significant differences were accepted for values of $p<0.05$. For the statistical analysis, half the value of the lower limit of each cytokine level was used as a substitute for values that were below the limit of detection.

III. Results

1. Body weights

The body weights of rats gradually increased, but did not significantly change between the 15-week time point and the endpoint (15-week-old rats, 432 ± 8.7g; endpoint, 482 ± 11g). The increase in body weight during this time did not significantly

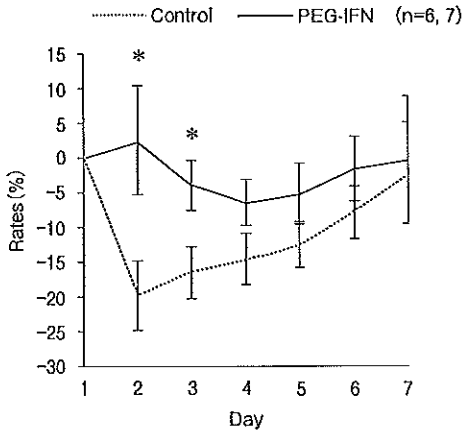

Fig. 2. Decreasing rates of the daily nighttime locomotor activity (LA) in the first week (n=24). Rats were injected pegylated interferon (PEG-IFN, n=12) or control (n=12). Decreasing rates from day 1 to day 2 and 3 were significantly reduced in PEG-IFN compared with in control. The data are presented as the means ± SEM. *p<0.05 versus control.

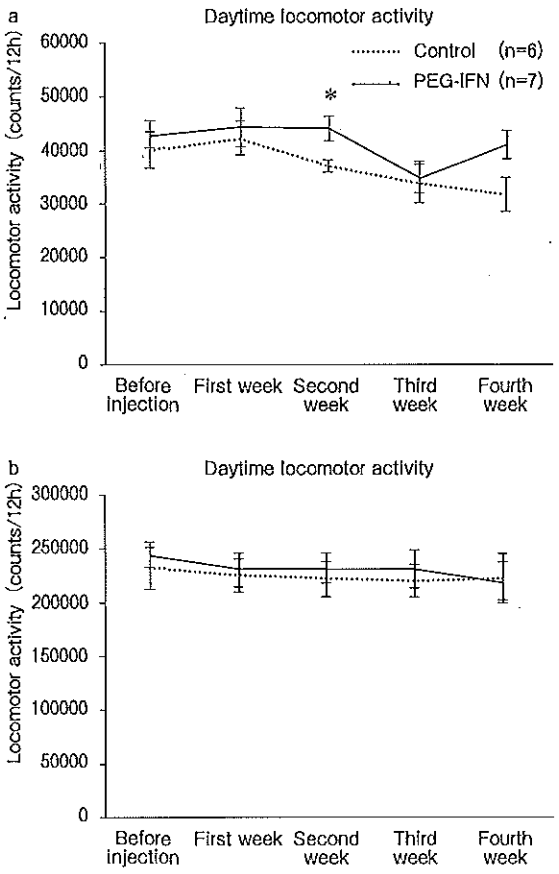

Fig. 3. a: The weekly daytime locomotor activity (LA) of rats injected with pegylated interferon (PEG-IFN) was significantly elevated during the second week compared with the control rats.
b: The weekly nighttime LA did not show any significant changes. The data are presented as the means ± SEM. *p<0.05 versus control.

differ between the treated and control rats (PEG-IFN- α 2b, 12 ± 0.47%; control, 12 ± 1.2%).

2. Daytime and nighttime LA in rats

The serial mean nighttime and daytime LA for every 12 hours of the PS group (control rats) showed no significant differences (data do not shown). In PEG-IFN- α 2b group, the daily nighttime LA during the first week showed no changes, while the PS group showed a significant decrease on days 2, 3, 4, 5 and 6 after administration. The decreasing rate of nighttime LA from the first day (day 1) to the second and third days (days 2 and 3) during the first week were significantly decreased in the PS group compared with the PEG-IFN- α 2b group (PS group: −20 ± 5.1% and −17 ± 3.7%, PEG-IFN- α 2b group: 2.6 ± 7.7% and −3.9 ± 3.7%, respectively) (Fig. 2). Additionally, the weekly daytime LA of the PEG-IFN- α 2b group had a tendency to be higher compared with that of the PS group during the entire 4-week period. In particular, significant differences in activity were noted in the second week (PEG-IFN- α 2b, 44052 ± 2189 counts; Control, 36894 ± 1085 counts, $p<0.05$). On the other hand, the weekly nighttime LA did not show significant changes in either group (Fig. 3a and b).

3. Immobility time during the FST

The immobility time during the FST was significantly increased at the point of 24 hours

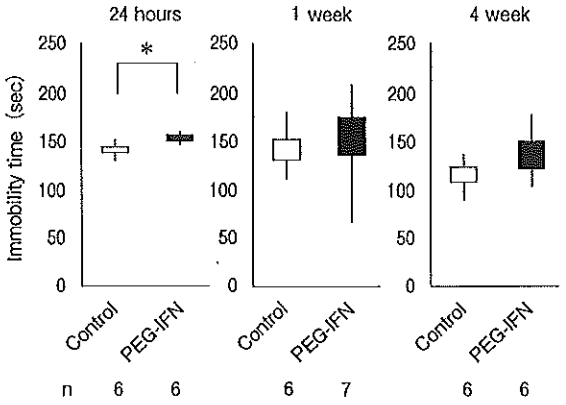

Fig. 4. The immobility time in the forced swim test (FST). At the time point 24 hours after administration, the immobility time was significantly increased in the rats injected with pegylated interferon (PEG-IFN). But, the immobility time did not show any significant differences one and 4 weeks after the start of treatment. The data are presented as the means ± SEM. *p=0.01 versus control.

Table 1. Cytokine levels in serum of rats sacrificed 24 hours after treated with a single injection without FST

Cytokine	Serum	
	Control	PEG-IFN- α 2b
IL-1α	1102 ± 74.36	1410 ± 288.2
IL-1β	3786 ± 129.7	3470 ± 400.8
IL-2	6166 ± 334.9	7003 ± 1228
IL-4	656.6 ± 7.635	674.8 ± 86.32
IL-5	2004 ± 77.31	1950 ± 128.4
IL-6	6255 ± 362.6	6376 ± 918.7
IL-10	5697 ± 299.1	7229 ± 1944
IL-12	585.1 ± 31.11	583.2 ± 63.98
IL-13	524.2 ± 27.04	560.2 ± 82.24
GM-CSF	2982 ± 34.93	2899 ± 84.59
IFN-γ	1114 ± 65.08	1249 ± 217.6
TNF-α	421.5 ± 52.25	470.3 ± 86.83

FST: forced swim test, PEG-IFN: pegylated interferon
The data are presented as the means ± SEM (pg/ml)

after the first administration of PEG-IFN- α 2b (PEG-IFN- α 2b group, 153 ± 3 seconds; PS group, 141 ± 3 seconds, p=0.01), but not significantly changed at the other time points after 1 week and 4 weeks of treatment (Fig. 4).

4. Influence of a single administration of PEG-IFN- α 2b on the cytokine levels in rats

After the administration of PEG-IFN- α 2b or PS, we evaluated the cytokine levels in rats sacrificed without undergoing the FST at the 24-hour time point when the depressive-like behavior was observed as an increased immobility time in the rats undergoing the FST. The serum cytokine levels in rats administered PEG-IFN- α 2b without the FST are shown in Table 1. The serum cytokine levels were not significantly different between the PS and PEG-IFN- α 2b groups. On the other hand, the levels of TNF- α , IL-2, IL-5, IL-12 and IL-13 in several regional brain tissues were significantly decreased, but there were no apparent tendencies in the relationship of the decrease in each cytokine and the specific region of the brain (Table 2).

In rats subjected to the FST, the serum levels of IL-1 α , IL-2, IL-4, IL-5, IL-6, IL-10, IL-13 and IFN- α were significantly decreased at 24 hours (Table 3). The cytokine levels in the frontal cortex, as a representative brain tissue, are shown in Table 4. All cytokines levels in the PEG-IFN- α 2b group showed a tendency to be decreased compared with those with in the PS group, and IL-13 and TNF- α were significantly lower.

IV. Discussion

PEG-IFN- α 2a and 2b are key drugs used for the standard anti-viral therapy for CHC patients. These drugs often induce

Table 2. Cytokine levels in brain of rats sacrificed 24 hours after treated with a single injection without FST

Cytokine	Frontal cortex		Temporal cortex		Parietal cortex		Occipital cortex	
	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b
IL-1α	0.82±0.80	0.37±0.28	0.90±0.64	0.86±0.84	1.3±0.76	1.3±0.32	0.02±0	0.50±0.40
IL-1β	0.4±0.33	1.6±1.4	0.070±0	0.070±0	0.47±0.32	1.1±0.92	2.4±2.4	1.3±1.2
IL-2	0.02±0	0.020±0	0.020±0	0.020±0	2.3±0.74	3.7±2.1	0.19±0.17	1.2±1.2
IL-4	0.78±0.38	0.22±0.13	0.26±0.26	0.035±0.035	0.17±0.17	0.035±0.035	0.23±0.23	0.16±0.12
IL-5	10±4.1	14±4.0	16±6.3	20±2.4	18±3.6	3.1±1.8*	12±4.0	19±3.1
IL-6	0.5±0.30	0.66±0.46	1.1±0.43	1.2±1.0	0.20±0	0.20±0	0.83±0.63	0.22±0.020
IL-10	58±24	47±17	18±11	37±12	38±17	17±9.4	17±5.7	27±16
IL-12	2.7±2.6	0.44±0.34	2.0±1.1	0.65±0.55	4.6±1.4	4.4±1.5	5.8±2.9	0.17±0.065
IL-13	1.8±0.40	0.25±0.053*	1.1±0.51	0.20±0.15	0.37±0.35	0.57±0.55	1.0±0.50	0.020±0*
GM-CSF	6.3±0.21	7.4±0.43	8.5±0.24	6.6±0.26	5.5±0.19	7.8±0.18	7.5±0.43	8.3±0.42
IFN-γ	12±4.5	2.1±1.2	2.3±2.1	5.1±4.9	0.18±0	0.18±0	2.0±1.8	2.8±2.0
TNF-α	1.0±0.37	0.020±0*	0.43±0.24	0.020±0*	0.24±0.22	0.020±0*	0.020±0	0.020±0

Cytokine	Stem		Cerebellum		Hippocampus		Pituitaly	
	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b
IL-1α	0.67±0.57	0.26±0.25	2.6±1.1	0.39±0.17	0.020±0	0.95±0.93	0.020±0	0.020±0
IL-1β	3.3±1.7	2.2±2.0	8.1±2.7	1.4±1.4	5.0±2.6	1.3±1.2	0.070±0	0.070±0
IL-2	4.9±1.9	1.5±1.1	3.3±0.83	0.020±0*	2.2±1.0	0.020±0*	0.020±0	0.020±0
IL-4	0.28±0.28	0.19±0.19	0.62±0.35	0.035±0.035	0.24±0.14	0.015±0.015	0.83±0.40	0±0*
IL-5	8.6±3.6	5.2±2.7	14±4.6	9.6±2.2	11±4.3	15±2.6	11±3.8	16±5.5
IL-6	1.2±0.67	0.20±0*	0.99±0.79	0.48±0.16	0.26±0.060	1.2±0.74	0.96±0.51	0.20±0*
IL-10	5.8±5.1	23±19	16±9.7	27±17	15±10	27±19	19±8.6	8.4±7.6
IL-12	8.1±4.1	1.0±0.90	9.6±3.5	1.4±1.1	9.2±1.1	2.4±2.2*	1.1±1.0	0.10±0*
IL-13	1.8±0.83	0.46±0.41	0.81±0.42	0.045±0.025	0.81±0.64	0.59±0.35	0.50±0.48	0.16±0.16
GM-CSF	6.9±0.048	3.7±0.35	5.7±0.36	3.4±0.23	7.2±0.087	7.8±0.32	3.3±0.28	2.2±0.24
IFN-γ	14±6.9	3.3±3.2	4.4±4.2	1.8±0.95	7.4±3.9	9.9±5.6	2.7±2.5	4.8±3.0
TNF-α	0.020±0	0.020±0	0.24±0.22	0.40±0.38	0.24±0.22	0.020±0*	0.11±0.090	0.16±0.14

FST: forced swim test, PEG-IFN: pegylated interferon
*$p<0.05$ control (n=4) versus PEG-IFN-α 2b administration (n=4)
The data are presented as the means ± SEM (pg/mg protein)

psychiatric symptoms such as depression and/or sleep disturbance as severe side effects. However, the mechanisms underlying these symptoms are still unclear. In the present study, we focused on the relationship between the cytokine levels in blood and brain tissues and psychiatric behaviors using the LA and the FST in normal rats with the same dosage of PEG-IFN-α 2b as administered in CHC patients. Because it is considered that LA abnormalities would represent sleep disturbance and/or irritability in rats, we evaluated the total serial daytime activities and nighttime activities every 12 hours during experimental periods and compared them between rats with or without PEG-IFN-α 2b treatment. On the other hand, as the immobility time in the FST is represented as a "depressive-like" status, we measured at the points of 24 h, 1 week, and the end of 4 weeks

Table 3. Cytokine levels in serum of rats sacrificed after FST

	24 hours		1 week		4 weeks	
	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b
n	6	6	6	6	6	6
IL-1α	548 ± 170	70 ± 23*	983 ± 198	623 ± 188	965 ± 348	92 ± 16
IL-1β	1612 ± 589	99 ± 38	2861 ± 630	2231 ± 814	2257 ± 711	579 ± 320
IL-2	3396 ± 796	679 ± 218*	5131 ± 909	3654 ± 946	4465 ± 1463	948 ± 184
IL-4	339 ± 121	33 ± 10*	539 ± 112	378 ± 116	342 ± 130	53 ± 13
IL-5	1166 ± 298	297 ± 76*	2314 ± 751	1201 ± 270	1041 ± 372	391 ± 48
IL-6	2820 ± 968	306 ± 79*	5166 ± 1135	3164 ± 1009	4090 ± 1338	394 ± 67
IL-10	3541 ± 820	877 ± 205*	4902 ± 897	3550 ± 843	4611 ± 1638	982 ± 235
IL-12	261 ± 96	22 ± 8.6	515 ± 117	338 ± 109	309 ± 127	29 ± 11
IL-13	264 ± 93	13 ± 7.2*	425 ± 91	298 ± 102	278 ± 118	29 ± 10
GM-CSF	362 ± 133	13 ± 4.9	622 ± 142	402 ± 140	474 ± 145	19 ± 4.6
IFN-γ	1050 ± 288	916 ± 265	1565 ± 464	1181 ± 201	5425 ± 4717	1292 ± 219
TNF-α	183 ± 63	13 ± 8.1*	376 ± 88	183 ± 82	202 ± 89	8.2 ± 4.3

FST: forced swim test, PEG-IFN: pegylated interferon
*$p<0.05$ control versus PEG-IFN- α2b administration
The data are presented as the means ± SEM (pg/ml)

Table 4. Cytokine levels in frontal cortex of rats sacrificed after FST

	24 hours		1 week		4 weeks	
	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b	Control	PEG-IFN-α2b
n	6	6	6	6	6	6
IL-1α	1.3 ± 1.2	0.12 ± 0	2.1 ± 0.7	1.1 ± 0.4	3.2 ± 1.4	13 ± 2.1*
IL-1β	1.6 ± 1.5	0.12 ± 0	4.7 ± 1.4	1.5 ± 0.8	13 ± 7.5	33 ± 6.9
IL-2	1.7 ± 1.1	0.02 ± 0	34 ± 5	14 ± 5.6*	77 ± 44	111 ± 44
IL-4	0.18 ± 0.1	0 ± 0	1.6 ± 0.1	0.88 ± 0.2*	2.5 ± 0.8	3.4 ± 1.2
IL-5	13 ± 3.2	6.1 ± 1.1	18 ± 1.4	8.4 ± 2.9*	12 ± 7.2	36 ± 13
IL-6	1.6 ± 1.4	0.2 ± 0	11 ± 1.6	4.7 ± 1.5*	27 ± 13	24 ± 7.3
IL-10	36 ± 11	12 ± 6.5	30 ± 4.3	23 ± 5.5	47 ± 15	88 ± 28
IL-12	2.7 ± 2.2	1.3 ± 1.2	0.72 ± 0.3	0.19 ± 0.1	0.88 ± 0.5	3.5 ± 0.6*
IL-13	0.18 ± 0.1	0.08 ± 0	0.62 ± 0.4	0.27 ± 0.2	2.3 ± 1.2	4.8 ± 1.8
GM-CSF	0.89 ± 0.5	0.06 ± 0	1.8 ± 0.9	0.18 ± 0.1*	2.1 ± 0.8	1.4 ± 0.7
IFN-γ	2.7 ± 2.6	0.18 ± 0	11 ± 2.2	6.6 ± 2.2	36 ± 15	39 ± 24
TNF-α	0.63 ± 0.4	0.2 ± 0	3.3 ± 1.5	1.1 ± 0.3	2.9 ± 1.8	2.6 ± 1.6

FST: forced swim test, PEG-IFN: pegylated interferon
*$p<0.05$ control versus PEG-IFN- α2b administration
The data are presented as the means ± SEM (pg/mg protein)

after the final PEG-IFN- α 2b administration. As shown the results, we first found that the subcutaneous administration of PEG-IFN- α 2b during the 4-week period induced abnormalities of the LA. Interestingly, the total counts for the daytime LA in the PEG-IFN- α 2b group were maintained compared with that of the PS group, and at the second week there was a significant difference (Fig. 4). On the other hand, although the total counts

for the nighttime LA showed no significant differences between the PEG-IFN- α 2b and PS groups, significant differences were found in the early days after PEG-IFN- α 2b or PS administration (Fig. 3). These results suggest that phenomena such as a decrease in sleeping or resting times might be transient, and might recover during the continuous administration of PEG-IFN- α 2b. Additionally, PS injection might also have affected the behavior of the rats. The onset of psychiatric symptoms in CHC patients does not parallel the administration period of PEG-IFN- α 2b. In addition, many patients who receive anti-viral therapy have other problems and are reported to have a disturbed quality of life [19]. Therefore, our data also suggest that the sensitivity to PEG-IFN- α 2b may be different for individual rats or between different strains of rats. Further studies are needed to evaluate the relationships among the dosage and duration of administration of PEG-IFN- α 2b and the daily LA.

The immobility time during the FST was significantly longer at 24 h after first PEG-IFN- α 2b administration, but not at 1 week and 4 weeks after treatment. It has been previously reported that rodents treated with human non-pegylated IFN- α subcutaneously or intravenously showed depressive-like behavior in the FST, tail suspension test and sucrose preference test [13-15]. Concerning the relationship between psychiatric symptoms and PEG-IFN- α treatment, previous experimental studies have shown that PEG-IFN- α 2a or 2b using the same dosage for human subjects did not influence the psychiatric behaviors such as reward behaviors, sickness or depressive-like behavior in rodents [20-22]. However, in these studies, the PEG-IFN- α 2a or 2b was administered intraperitoneally. When substances including PEG-IFN- α 2a or 2b are administered intraperitoneally, they are mainly absorbed into the portal vein and metabolized in the liver [23]. Approximately 70% of PEG-IFN- α 2b is cleared through hepatic catabolism and degeneration [24, 25]. Therefore, the effects of PEG-IFN- α 2a or 2b might be decreased in such cases, despite the long half-life of the compound, unless they are given frequently to provide a higher serum concentration. In contrast, PEG-IFN- α 2a or 2b administered subcutaneously is slowly absorbed into the circulation and shows a long-term effect. These previous studies and our present study suggest that the route of administration may be a factor associated with the appearance of abnormal psychiatric behavior induced by PEG-IFN- α 2a or 2b administration. On the other hand, it has been reported that the hepatitis C virus status influences the function of the brain directly or indirectly [26]. As many previous studies and our present study have used rats or mice without chronic liver injury, in the future it will be necessary to clarify the effect of PEG-IFN- α 2a or 2b against the LA and depressive-like behavior in models with chronic liver injury.

Many cytokines that are induced or reduced by IFN administration have been closely associated with the disturbance of functions in the CNS [3, 5]. Thus, in the present study, we measured the levels of 12 cytokines in the serum and brain of rats under conditions such as 1) with or without PEG-IFN- α 2b, 2) with or without the FST. As shown in the results, there were two new findings: 1) a

single subcutaneous injection of PEG-IFN- α 2b without the FST induced a decrease in the brain cytokine levels, but not in the serum levels and 2) the FST can affect the levels of serum and brain cytokines. In the present study, although we measured the cytokine levels in the serum and brain tissues in rats sacrificed 1 and 4 weeks after treatment, we did not show the results, because the cytokine levels might have been influenced by the FST. Therefore, to clarify the direct effects of the long-term administration of PEG-IFN- α 2b on the serum and brain cytokine levels, it will be necessary to plan an experiment without the FST.

There is no significant correlation between the levels of cytokines in serum and brain tissues in this experimental study. When PEG-IFN- α 2a or 2b are administered subcutaneously, these IFNs are distributed into many tissues or organs including the brain. Therefore, it is considered that in the brain IFN transferred through the BBB acts directly on microglia and/or astrocytes. Although the reason is not clear at present, it is considered that the small number of samples, low concentrations of measured cytokines and the differences in the production and catabolism of cytokines among each tissue are related to this discrepancy. To evaluate the cytokine network and relationships between blood and brain tissue, further studies, including the dose, site of administration, frequency of administration and total dose administered, time course of experiments, and different species will be needed in the future.

In conclusion, the present study indicates that the locomotor activity and behavior in normal rats might be disturbed during the early stage of the subcutaneous administration of PEG-IFN- α 2b or even just a stress load, such as PS injection. The cytokines levels in both the serum and brain tissues are also influenced by the administration of PEG-IFN- α 2b and the FST, although further studies will be needed to clarify the association between the cytokine changes and the onset of psychiatric symptoms.

Acknowledgments

We thank Dr. Hiroki Oikawa and Professor Tomoyuki Masuda for valuable advice on our experiments. We also thank Ms. Asako Watanabe for her technical assistance.

This study was supported by the Japan Society for the Promotion of Science and a Grant-in-Aid for Scientific Research (No. 21590857).

Conflict of interest: The authors have no conflict of interest to declare.

References

1) **Dianzani F, Antonelli G** and **Capobianchi MR**: The biological basis for clinical use of interferon. J Hepatol **11** (Suppl 1), S5-10, 1990.
2) **Malaguarnera M, Di Fazio I, Restuccia S, et al.**: Interferon alpha-induced depression in chronic hepatitis C patients: comparison between different types of interferon alpha. Neuropsychobiology **37**, 93-97, 1998,.
3) **Wichers M** and **Maes M**: The psychoneuroimmunopathophysiology of cytokine-induced depression in humans. Int J Neuropsychopharmacol **5**, 375-388, 2002.
4) **Reyes-Vazquez C, Prieto-Gomez B** and **Dafny N**: Interferon modulates central nervous system function. Brain research **1442**, 76-89, 2012.
5) **Licinio J, Kling MA** and **Hauser P**: Cytokines and brain function: relevance to interferon-alpha-induced mood and cognitive changes. Semin

Oncol **25**, 30-38, 1998.

6) **Taylor JL** and **Grossberg SE**: The effects of interferon-alpha on the production and action of other cytokines. Semin Oncol **25**, 23-29, 1998.
7) **Brassard DL, Grace MJ** and **Bordens RW**: Interferon-alpha as an immunotherapeutic protein. J Leukoc Biol **71**, 565-581, 2002.
8) **Brandt ER, Mackay IR, Hertzog PJ, et al.**: Molecular detection of interferon-alpha expression in multiple sclerosis brain. J Neuroimmunol **44**, 1-5,1993.
9) **Khan NU, Pulford KA, Farquharson MA, et al.**: The distribution of immunoreactive interferon-alpha in normal human tissues. Immunology **66**, 201-206, 1989.
10) **Yamada T** and **Yamanaka I**: Microglial localization of alpha-interferon receptor in human brain tissues. Neurosci Lett **189**, 73-76, 1995.
11) **Wang T, Takikawa Y, Sawara K, et al.**: Negative regulation of human astrocytes by interferon (IFN) alpha in relation to growth inhibition and impaired glucose utilization. Neurochem Res **37**, 1898-1905, 2012.
12) **Takeda H, Tsuji M, Ikoshi H, et al.**: Effects of a 5-HT7 receptor antagonist DR4004 on the exploratory behavior in a novel environment and on brain monoamine dynamics in mice. Eur J Pharmacol **518**, 30-39, 2005.
13) **Yamano M, Yuki H, Yasuda S, et al.**: Corticotropin-releasing hormone receptors mediate consensus interferon-alpha YM643-induced depression-like behavior in mice. J Pharmacol Exp Ther **292**, 181-187, 2000.
14) **Makino M, Kitano Y, Komiyama C, et al.**: Human interferon-alpha increases immobility in the forced swimming test in rats. Psychopharmacology (Berl) **148**, 106-110, 2000.
15) **Ping F, Shang J, Zhou J, et al.**: 5-HT(1A) receptor and apoptosis contribute to interferon-alpha-induced "depressive-like" behavior in mice. Neurosci Lett **514**, 173-178, 2012.
16) **Porsolt RD, Le Pichon M** and **Jalfre M**: Depression: a new animal model sensitive to antidepressant treatments. Nature **266**, 730-732, 1977.
17) **Cryan JF, Hoyer D** and **Markou A**: Withdrawal from chronic amphetamine induces depressive-like behavioral effects in rodents. Biol Psychiatry **54**, 49-58, 2003.
18) **Hulse RE, Kunkler PE, Fedynyshyn JP, et al.**: Optimization of multiplexed bead-based cytokine immunoassays for rat serum and brain tissue. J Neurosci Methods **136**, 87-98, 2004.
19) **Mathew A, Peiffer LP, Rhoades K, et al.**: Improvement in quoloty of life measures in patients with refractory hepatitis C, responding to re-treatment with pegylated interferon alpha-2b and ribavirin. Health Qual Life Outcomes **4**, 30, 2006
20) **De La Garza R, 2nd, Asnis GM, Pedrosa E, et al.**: Recombinant human interferon-alpha does not alter reward behavior, or neuroimmune and neuroendocrine activation in rats. Prog Neuropsychopharmacol Biol Psychiatry **29**, 781-792, 2005.
21) **Loftis JM, Wall JM, Pagel RL, et al.**: Administration of pegylated interferon-alpha-2a or -2b does not induce sickness behavior in Lewis rats. Psychoneuroendocrinology **31**, 1289-1294, 2006.
22) **Kosel M, Bilkei-Gorzo A, Zawatzky R, et al.**: Pegylated human interferon alpha 2a does not induce depression-associated changes in mice. Psychiatry Res **185**, 243-247, 2011.
23) **Turner PV, Brabb T, Pekow C, et al.**: Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci **50**, 600-613, 2011.
24) **Zeuzem S, Welsch C** and **Herrmann E**: Pharmacokinetics of peginterferons. Semin Liver Dis **23** (Suppl 1), 23-28, 2003.
25) **Cai Y, Zhang Z, Fan K, et al.**: Pharmacokinetics, tissue distribution, excretion, and antiviral activity of pegylated recombinant human consensus interferon-alpha variant in monkeys, rats and guinea pigs. Regul Pept **173**, 74-81, 2012.
26) **Schaefer M, Capuron L, Friebe A, et al.**: Hepatitis C infection, antiviral treatment and mental health: A European expert consensus statement. J Hepatol **57**, 1379-1390, 2012.

岩手医誌 66 巻, 3 号（平成 26 年 8 月）101-112 頁.

pegylated interferon α -2b 皮下投与による ラットの行動と血清および脳内サイトカインへの影響

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(Received on January 23, 2014 & Accepted on January 26, 2014)

要旨

pegylated interferon (PEG-IFN) によりうつ等の精神症状が生じるが, その機序は明らかではない. 我々は週 1 回 PEG-IFN α -2b を 4 週間ラットに皮下投与し, 自発行動活性, 強制水泳試験での無動時間, 血中および脳内のサイトカインへの影響を検討した. PEG-IFN α -2b 投与群で第 2 週の昼間行動活性が上昇した. 第 1 週の夜間行動活性は対照群で有意に減少したが, PEG-IFN α -2b 投与群では 2, 3 日目の減少率が低下した. 強制水泳試験での無動時間は初回投与後 24 時間で延長を認めた. 初回投与後 24 時間のラット脳内では複数のサイトカインの減少が見られたが血清での変動は認めず, サイトカインの血清と脳内の動態は同一の傾向を認めなかった. ラットは皮下投与後の早期の段階で PEG-IFN α -2b の影響を受け, 自発行動活性, 強制水泳試験, サイトカイン濃度が変動すること示唆された.