0022-3565/97/2831-0259$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 283, No. 1

Printed in U.S.A.
JPET 283:259–264, 1997

Chronopharmacological Study of Interferon-α in Mice

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Accepted for publication June 12, 1997

ABSTRACT

The influence of dosing time on the pharmacological effects (fever and antiviral activity) and the pharmacokinetics of interferon-α (IFN-α) was investigated in ICR male mice under light-dark (12:12) cycle. There was a significant circadian rhythm in rectal temperature, as an index of fever, at 0.5 hr after IFN-α (10.0 MIU/kg i.v.) injection. The rhythmic pattern resembled overall the rhythm that occurs in the nondrugged state. However, the percent change from basal level of rectal temperature varied according to the dosing time. The rhythmicity corresponded to the dosing time-dependent difference of PGE_2 levels in thalamus after IFN-α injection, but it did not correspond to that of plasma IFN-α concentrations. A significant dosing time-dependent difference was also demonstrated for 2′-5′oligoadenylate synthetase activities, as an index of antiviral activity, in plasma and liver at 24 hr after IFN-α injection. It was related to the rhythmicity in plasma IFN-α concentrations that was caused by the rhythmicity in clearance of IFN-α. The choice of the most appropriate time of day for drug administration may help to achieve rational chronotherapeutics of IFN-α in certain experimental and clinical situations.

A large number of physiological rhythmic variables are demonstrated in the CNS, in hormone secretion and so on (Kafka *et al.*, 1981; Naber *et al.*, 1981; Thomson *et al.*, 1980). Also, many drugs vary in potency and/or toxicity according to the time in the circadian cycle when they are administered (Ohdo *et al.*, 1988, 1990, 1995a, 1995b, 1996; Frederickson *et al.*, 1977; Walker and Owasoyo, 1974).

Interferons, which belong to a group of cytokines, have been widely used as antiviral and antitumor agents in the human. However, interferons cause unavoidable adverse effects such as fever, fatigue, headache, rigors and myalgias. In particular, fever is an indispensable side effect in nearly all patients during the early phase of interferon treatment. Administration of IFN-α in cancer patients is better tolerated in evening than in morning (Abrams *et al.*, 1985). There are also significant dosing time-dependent differences in the antitumor and myelosuppressive activity of IFN-α in mice (Koren *et al.*, 1993; Koren and Fleischmann, 1993). However, the rhythmic changes of interferon-induced fever and antiviral activity have not yet been examined.

Rectal temperature and immune functions show significant circadian rhythms in mammals under both nondrugged and drugged conditions (Ohdo *et al.*, 1995a; Refinetti *et al.*, 1990; Haus *et al.*, 1983; Batalla *et al.*, 1994). Therefore, there may be a chronobiologic effect on the fever and antiviral activity induced by IFN-α. The increase in body temperature induced by interferons may be one aspect of its antiviral activity as an immunoadjuvant effect, but an excessive febrile reaction may be more detrimental than beneficial.

The purpose of this study was to examine the diurnal change of IFN-α induced fever and antiviral activity in mice. The mechanisms underlying these phenomena were also investigated from the perspective of IFN-α pharmacokinetics.

Methods

Animals and treatments. Male ICR mice (5 weeks old) were purchased from Charles River Japan Inc. (Kanagawa, Japan). Mice were housed 6 or 10 per cage in a light-controlled room (light on from 07:00 to 19:00) at a room temperature of 24°C ± 1°C and a humidity of 60% ± 10% with food and water *ad libitum*. All mice were adapted to their light-dark cycle for 2 weeks before the experiments. In order to study the fever induced by IFN-α (Sumiferon, Sumitomo Seiyaku Co., Osaka, Japan), groups of six mice injected i.v. with 1.0, 5.0 or 10.0 MIU/kg IFN-α or sterilized saline at the same circadian phase (09:00). IFN-α was diluted by sterilized saline to adjust the concentration to 0.2, 1.0 and 2.0 MIU/ml. The volume of injection was 0.05 ml/10.0 g b.wt. The drug solutions were used within 30 min after preparation in order not to decrease their biologic activity. Rectal temperature was continuously determined before, and at 0.5, 1.0, 2.0 and 4.0 hr after, IFN-α or saline injection. In the study of the circadian rhythms of IFN-α-induced fever and plasma IFN-α concentrations, groups of 8 to 10 mice were injected i.v. with 10.0 MIU/kg IFN-α or saline at one of six times: 09:00, 13:00, 17:00, 21:00, 01:00 or 05:00. Rectal temperature was determined before, and at 0.5, 1.0, 1.5 and 2.0 hr after, IFN-α or saline injection. Percent change of rectal temperature (%) from basal level was calculated as follows: % = ([rectal temperature after IFN-α injection − rectal IFN-α before

Received for publication December 27, 1996.

ABBREVIATIONS: IFN-α, interferon-α; 2′-5′OAS, 2′-5′oligoadenylate synthetase; CL, clearance; V_c, central volume of distribution; K_{12}, distribution rate constant from central to peripheral compartment; K_{21}, distribution rate constant from peripheral to central compartment.

IFN-α injection] / [rectal temperature before IFN-α injection]) $\times$ 100. Blood samples were drawn by cardiac puncture at 2.5 hr after IFN-α injection and placed into polypropylene tubes containing 10 μl of EDTA (4%) solution. To observe the PGE_2 production induced by IFN-α, groups of six mice were injected i.v. with 10.0 MIU/kg IFN-α on one of two occasions: in the latter half of the light phase (17:00) or in the latter half of the dark phase (05:00). Blood samples were drawn by cardiac puncture at 0.5 hr after IFN-α injection and placed into polypropylene tubes containing 10 μl of indomethacin (40 mM) / EDTA (4%) solution. Immediately after blood sample collection, thalamus was removed and placed into ice-cold tubes. To examine 2′-5′OAS activities induced by IFN-α, groups of 8 to 10 mice were injected i.v. with 10.0 MIU/kg IFN-α on one of two occasions as described above. Blood samples were collected by cardiac puncture at 24 hr after IFN-α injection and placed into polypropylene tubes containing 10 μl of EDTA (4%) solution. Immediately after blood collection, liver was perfused with 0.01 M PBS. The liver was quickly removed, rinsed with saline and placed into ice-cold tubes. To study the time course of plasma IFN-α concentrations, groups of six mice were injected i.v. with 10.0 MIU/kg IFN-α on one of two occasions as described above. Blood samples were drawn by orbital sinus collection at 0.167, 0.5, 1.0, 2.0, 3.0 and 4.0 hr after IFN-α injection.

Determination of IFN-α induced fever. IFN-α induced fever was determined by measuring the rectal temperature after IFN-α or saline injection. Rectal temperature was measured on a digital thermometer (digital thermometer TD-300, Shibaura Electronics, Tokyo, Japan). A lubricated thermocouple was inserted 1.5 cm into the rectum of mice. Rectal temperature was measured at least every 30 min to avoid hyperthermia occasioned by continuous handling stress (Briese *et al.*, 1991).

Determination of PGE_2 concentration in plasma and thalamus. Plasma samples were obtained after centrifugation at 3000 rpm for 3 min. The plasma samples (300 μl) were added to ethanol solution to give a final concentration of 10% ethanol. The ice-cold thalamus was weighed and homogenized with the cold absolute ethanol (200 μl), distilled water being added to give final concentration of 10% ethanol. The supernatant, after centrifugation at 1500 $\times$ g $\times$ for 20 min, was used as the thalamus homogenate sample. PGE_2 was extracted from plasma and thalamus samples according to the method of Powell (Powell, 1982) and Shono (Shono *et al.*, 1988). The plasma and thalamus homogenate samples were acidified to pH 3.0 by acetic acid and applied to a SEP-PAK C_{18} column (Waters, Massachusetts). The solvent of crude extract was evaporated and dissolved in the HPLC mobile-phase buffer (methanol / H_2O/acetic acid, 60 / 40 / 0.01, v/v/v). Further purification was performed by HPLC using ODS-80Ts column (4.6 mm I.D. $\times$ 150 mm) connected to a pump (655A-11 Liquid Chromatograph, Hitachi, Tokyo, Japan). The flow rate was 0.8 ml/min. The PGE_2 fractions for assay were collected from 21 to 26 min. The solvent was evaporated, and the residue was dissolved in assay buffer (0.1% bovine serum albumin/0.1 M phosphate-buffered saline pH 7.4). PGE_2 concentrations were determined by enzyme immunoassay (PGE_2 immunoassay system, Amersham, Bukinghamshire, U.K.).

Determination of 2′-5′OAS activities in plasma and liver. Plasma samples were obtained after centrifugation at 3000 rpm for 3 min and then stored at −20°C until assayed. The ice-cold liver was immediately homogenized with modified lysis buffer (10 mM HEPES-KOH/50 mM KCl/3 mM $Mg(OAc)_2$/0.3 mM EDTA/10% glycerol/0.01% NaN_3/0.5% Triton-100/100 μM PMSF/7 mM 2-mercaptoethanol, pH 7.5) (Sokawa *et al.*, 1994). The supernatants, after centrifugation at 9000 $\times$ g for 20 min at 4°C, were used as the liver sample. The protein concentrations in the liver homogenate sample were determined by Lowry's method. The plasma and liver 2′-5′OAS activities were determined by radioimmunoassay (2-5A kit, Eiken, Tokyo, Japan). The 2′-5′OAS activities in liver were expressed as 2′-5′oligoadenylate fmol per liver protein concentration.

Determination of IFN-α concentration in plasma. Plasma samples were obtained after centrifugation at 3000 rpm for 3 min and stored at −20°C until assayed. Plasma IFN-α concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (IFN-α immunoassay kit, BioSource International Inc, California). The titer was expressed in international units (IU) per milliliter, and the detection limit in the sample was 10 IU/ml. There was no cross-reactivity with endogenous mouse interferons.

Statistical analysis. Pharmacokinetic parameters were calculated by the nonlinear least-squares method, following the two-compartment model: CL, V_c, K_{12} and K_{21}. Analysis of variance (ANOVA) and Tukey's test were applied for the multiple comparison. Student's t test was used for independent comparison between groups. The 5% level of probability was considered to be significant.

Results

Influence of IFN-α on body temperature. The effects of three dosages (1.0, 5.0 and 10.0 MIU/kg) of IFN-α on rectal temperature in mice injected with the drug at the same circadian phase (09:00) are shown in figure 1. The rectal temperature increased from basal level at all dosages of IFN-α. The rectal temperature at 0.5 hr after IFN-α 10.0 MIU/kg injection was significantly different from that after saline injection ($P < .01$). However, the rectal temperature of mice injected with IFN-α 1.0 or 5.0 MIU/kg was not significantly different from that of mice injected with saline.

Circadian rhythm of IFN-α induced fever. The rectal temperature in mice injected with saline showed significant circadian rhythm with a lower level during the light phase and a higher level during the dark phase ($P < .01$; fig. 2). The rectal temperature after IFN-α 10.0 MIU/kg injection was significantly higher during the 24-hr cycle when compared with that after saline injection ($P < .01$). The rhythmic pattern of IFN-α-induced fever resembled overall the rhythm that occurred after saline injection. However, fever was not induced by IFN-α injection in the latter half of the dark phase (05:00). The time course of rectal temperature was expressed as percent change from basal level, the level before IFN-α injection (fig. 3). The percent changes in rectal temperature

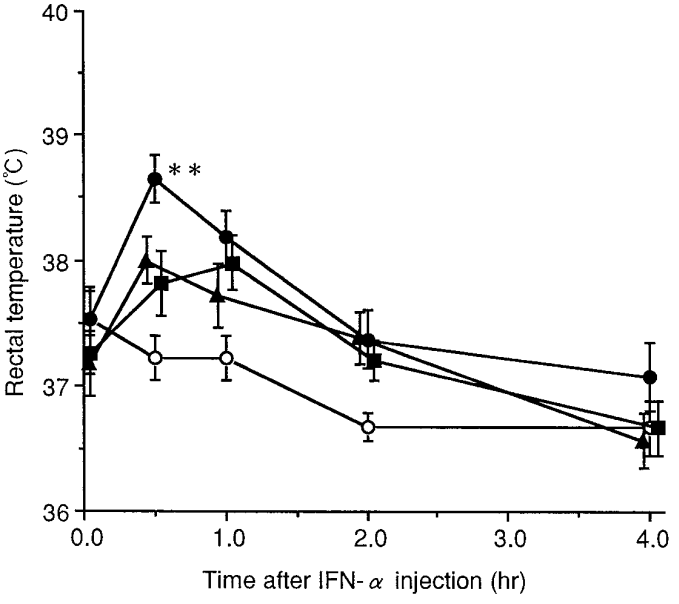

Fig. 1. The time course of rectal temperature after IFN-α injection. Each point represents the mean ± S.E. of six mice. ** $P < .01$ when compared with the saline group using Tukey's test. ○, saline; ●, IFN-α 10.0 MIU/kg; ■, 5.0 MIU/kg; ▲, 1.0 MIU/kg.

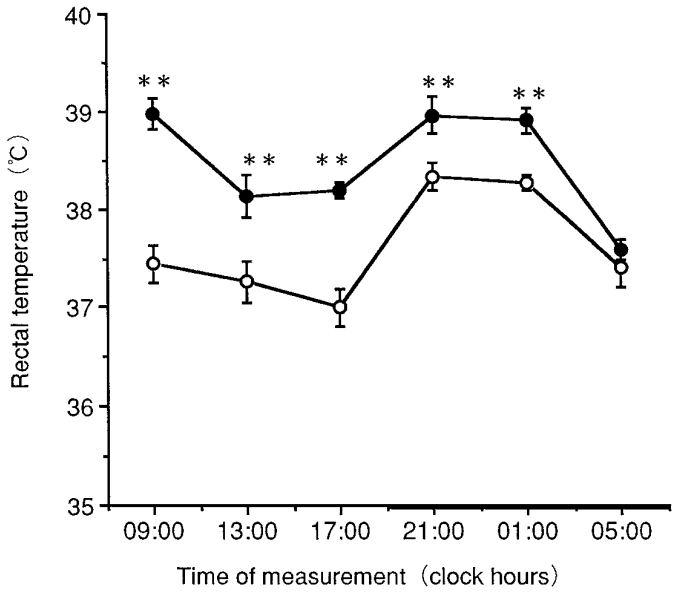

Fig. 2. Circadian rhythm of rectal temperature at 0.5 hr after IFN-α (10.0 MIU/kg i.v.) injection (●) or saline injection (○). Each point represents the mean ± S.E. of 8 to 10 mice. ** $P < .01$ when compared with the corresponding saline group using Tukey's test.

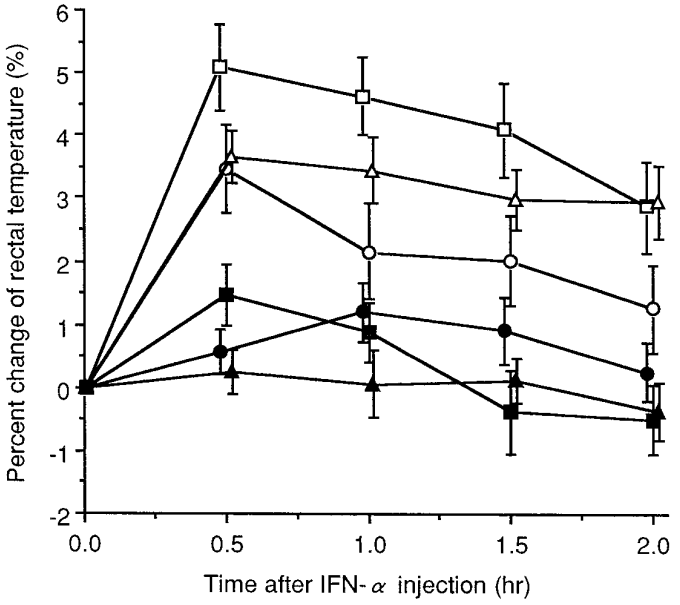

Fig. 3. The time course of percent change of rectal temperature after IFN-α (10.0 MIU/kg i.v.) injection at 09:00 (○), 13:00 (□), 17:00 (△), 21:00 (●), 01:00 (■) and 05:00 (▲). Value of rectal temperature is expressed as percent change from basal level. Each point represents the mean ± S.E. of 8 to 10 mice.

at 0.5 hr after IFN-α injection were significantly higher in the light phase than in the dark phase ($P < .01$). However, the rectal temperature after IFN-α injection in the latter half of dark phase (05:00) showed no significant difference from that after saline injection.

Influence of dosing time on PGE_2 levels in plasma and thalamus. The effect of dosing time of IFN-α on PGE_2 production in plasma and thalamus is shown in figure 4. There was no significant difference in PGE_2 levels in plasma and thalamus between mice injected with saline at 17:00 and 05:00. The PGE_2 levels in plasma after IFN-α injection at 17:00 tended to be higher than those after saline injection at 17:00 ($P < .10$). The PGE_2 levels in thalamus at 0.5 hr after IFN-α injection were significantly higher in mice injected with the drug at 17:00 than in those injected at 05:00 ($P < .01$). The PGE_2 levels in thalamus after IFN-α injection at 17:00 also increased significantly when compared with those after saline injection at 17:00 ($P < .01$).

Influence of dosing time on 2′-5′OAS activity in plasma and liver. The effect of time dosing with IFN-α on 2′-5′OAS activity in plasma and liver is shown in figure 5. 2′-5′OAS activities in plasma and liver showed no significant difference between mice injected with saline at 17:00 and 05:00. 2′-5′OAS activity in plasma at 24 hr after IFN-α injection was significantly higher for injection at 05:00 than for injection at 17:00 ($P < .05$), but 2′-5′OAS activity in liver at 24 hr after IFN-α injection showed no dosing time-dependent difference. 2′-5′OAS activities in plasma and liver after IFN-α injection at 05:00 increased significantly when compared with those after saline injection ($P < .01$, $P < .05$ respectively).

Circadian rhythm of IFN-α concentrations in plasma. The plasma IFN-α concentrations at 2.5 hr after IFN-α injection showed a significant circadian rhythm with higher levels from late dark phase to early light phase and lower levels from late light phase to early dark phase ($P < .01$; fig. 6). The time course of plasma IFN-α concentrations after IFN-α injection decayed biphasically (fig. 7). IFN-α concentrations at 2.0, 3.0 and 4.0 hr after IFN-α injection were significantly higher for injection at 05:00 than for injection at 17:00 ($P < .05$). CL was significantly higher in mice injected with IFN-α at 17:00 than in those injected at 05:00 ($P < .05$, table 1). There was no significant difference in any other pharmacokinetic parameters between mice injected with the drug at 17:00 and those injected at 05:00.

Discussion

Rectal temperature in mice showed a significant circadian rhythm with higher levels during the dark phase and lower levels during the light phase under nondrugged conditions. Our result confirms previous observations (Ohdo *et al.*, 1995a). Normal body temperature is regulated by the relative balance of catecholamine and serotonin levels in the anterior hypothalamus (Feldberg and Myers., 1964). The changes in locomotor activities, eating, drinking and secretion of several hormones influence the rhythm of rectal temperature (Refinetti and Menaker, 1992). The rectal temperature at 0.5 hr after IFN-α injection increased significantly during the 24-hr cycle, except for the latter half of the dark phase (05:00), when compared with that after saline injection. The rhythmic pattern of IFN-α-induced fever resembled overall the rhythm occurring after saline injection, but the percent changes from basal level of rectal temperature after IFN-α injection varied according to the dosing time. IFN-α acts on the thermosensitive neurons in the preoptic and anterior hypothalamus and increases body temperature *via* PGE_2 production and/or opioid receptor (Nakashima *et al.*, 1988, 1995; Dinarello *et al.*, 1984). Certainly, cyclooxygenase inhibitors, by decreasing PGE_2 production, suppress IFN-α induced fever. PGE_2 levels in the thalamus at 0.5 hr after IFN-α injection were significantly higher in mice injected with the drug at 17:00 than in those injected at 05:00. This

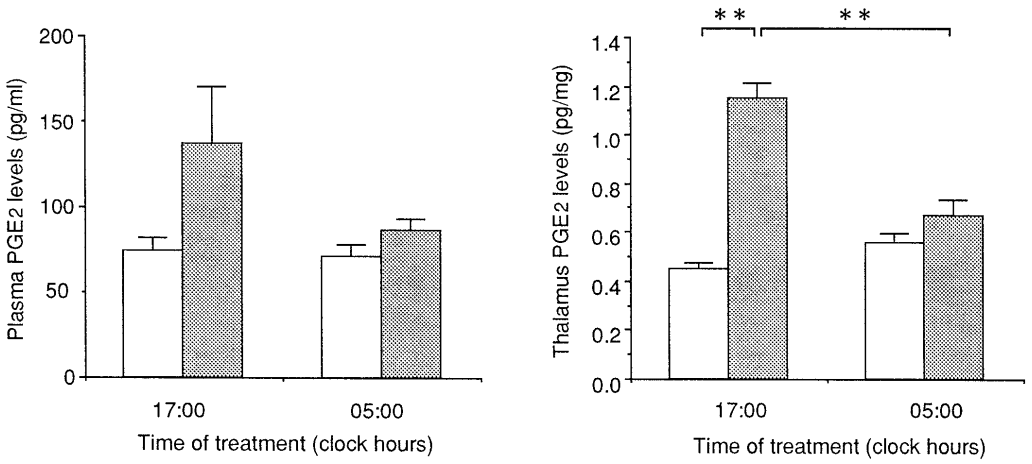

Fig. 4. Influence of dosing time on the plasma and thalamus PGE_2 levels after IFN-α (10.0 MIU/kg i.v.) injection at 17:00 or 05:00. Each column represents the mean ± S.E. of six mice. ** $P < .01$ when compared with the saline group or between the two dosing times using Tukey's test. □: saline; ▨: IFN-α.

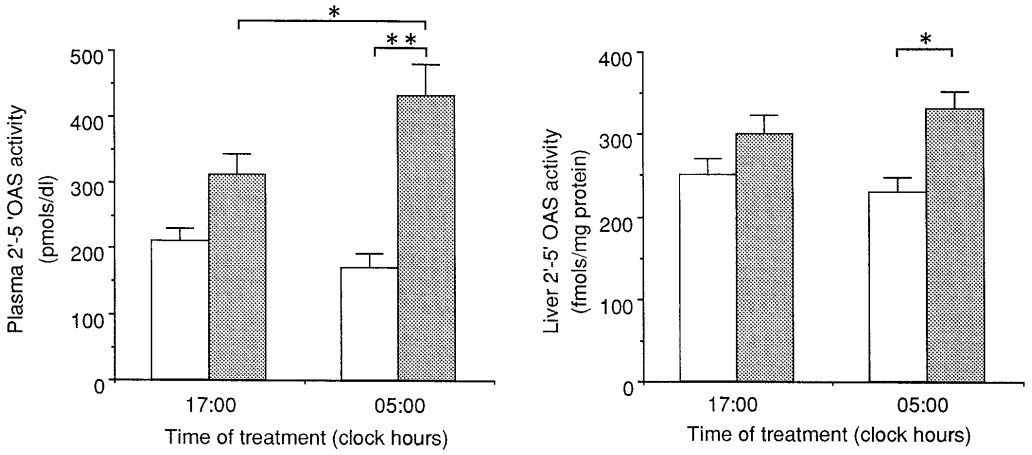

Fig. 5. Influence of dosing time on the plasma and liver 2′-5′OAS activities after IFN-α (10.0 MIU/kg i.v.) injection at 17:00 or 05:00. Each column represents the mean ± S.E. of 8 to 10 mice. * $P < .05$; ** $P < .01$ when compared with the saline group or between the two dosing times using Tukey's test. □: saline; ▨: IFN-α.

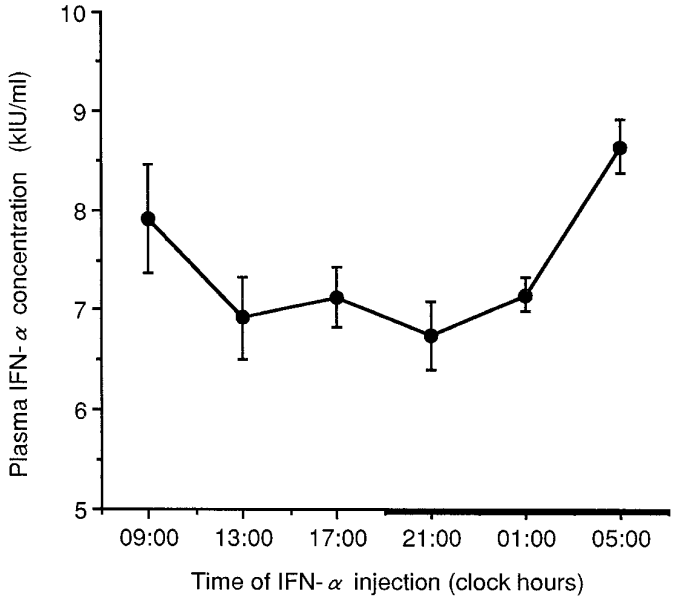

Fig. 6. Circadian rhythm of plasma IFN-α concentrations at 2.5 hr after IFN-α (10.0 MIU/kg i.v.) injection. Each point represents the mean ± S.E. of 8 to 10 mice.

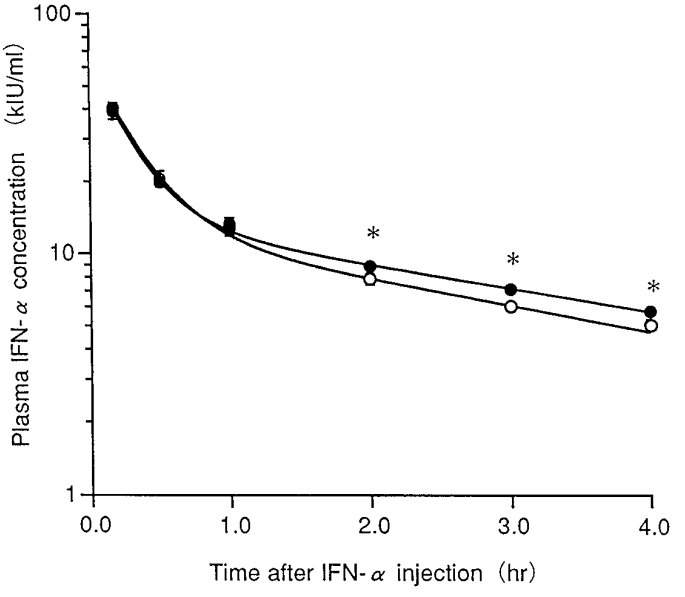

Fig. 7. The time course of plasma IFN-α concentrations after IFN-α (10.0 MIU/kg i.v.) injection at 17:00 (○) or 05:00 (●). Each point represents the mean ± S.E. of six mice. * $P < .05$ when compared between the two groups using Tukey's test.

seems to coincide with the circadian rhythm of IFN-α-induced fever. Although plasma IFN-α concentrations showed significant circadian rhythm, it was out of phase with the rhythm of IFN-α-induced fever. Thus the rhythmicity of IFN-α-induced fever seems to be due to that of the sensitivity of mice to the drug.

The important question still remains whether the antiviral activity of IFN-α declines at the dosing time that alleviates

TABLE 1

Influence of dosing time of IFN-α (10.0 MIU/kg i.v.) injection on pharmacokinetic parameters

Pharmacokinetic Parameter	Time of Injection (clock hours)		Statistical Significance
	17:00	05:00	
CL (L/hr/kg)	0.155 ± 0.008	0.125 ± 0.009	$P < .05$
Vc (L/kg)	0.177 ± 0.021	0.154 ± 0.025	N.S.
K12 (1/hr)	1.788 ± 0.299	2.620 ± 0.502	N.S.
K21 (1/hr)	0.973 ± 0.094	1.038 ± 0.118	N.S.

Values show mean ± S.E. of six mice. Statistical significance is compared between two groups by Student's *t* test.

IFN-α-induced fever. The antiviral activity of interferon due, at least in part, to the 2′-5′oligoadenylate synthetase system (Baglioni, 1979). 2′-5′OAS is the enzyme directly related to the antiviral action of interferon. Serum 2′-5′OAS activity is used as an index of the antiviral effect of interferon in patients with hepatitis. There was no significant dosing time-dependent difference in 2′-5′OAS activity between saline injection at 17:00 and that at 05:00. However, both plasma and liver 2′-5′OAS activities induced by IFN-α were higher in mice injected with drug at 05:00 than in those injected at 17:00. The rhythm corresponded well to the rhythmicity of IFN-α concentration. Therefore, the diurnal difference of 2′-5′OAS activity induced by IFN-α can be explained, at least in part, by the rhythm of plasma IFN-α concentration. The circadian rhythm of antitumor activity induced by IFN-α exhibits higher activity in the early light phase (Koren *et al.*, 1993). In the circadian phase, plasma IFN-α concentration was higher in the present study. The rhythm of IFN-α-induced antitumor activity also seems to be due to that of IFN-α pharmacokinetics.

Plasma IFN-α concentrations at 2.5 hr after IFN-α injection showed a significant circadian rhythm. A significant dosing time-dependent difference was also demonstrated for the pharmacokinetic parameter of IFN-α, which showed higher CL for injection at 17:00 than for injection at 05:00. The rhythmicity in CL seems to be closely related to that in plasma IFN-α concentration. IFN-α concentrations in plasma have been shown to decay biphasically after an i.v. injection of IFN-α, and the distribution phase lasted for 1.0 hr after the drug injection (Cantell and Pyhärä, 1973). IFN-α is quickly eliminated from the body by several pathways. The main route of excretion of IFN-α is the kidneys (Bino *et al.*, 1982). Renal tubular cells take up and break down many plasma proteins (Strober and Waldmann, 1974). IFN-α is also internalized and catabolized intracellularly in kidney *via* receptor-mediated endocytosis (Bocci *et al.*, 1983). Both renal elimination rate and liver metabolism rate increase during the active period in mice (Ohdo *et al.*, 1995a). The rhythmicity can reflect not only the rhythmic activity of the enzyme in kidney and liver but also the rhythmic rate of blood flow (Labrecque *et al.*, 1988). The rhythmicity of CL in our study corresponds well to that of blood flow. Thus the circadian rhythm in IFN-α pharmacokinetics may be caused by the diurnal rhythm of renal function. Although the circadian rhythm of receptor-mediated endocytosis has not been investigated yet, this should be clarified in future.

The present findings in this mouse model support the concept that the choice of the most appropriate time of day for administration of interferons may reduce their side effects and increase their antiviral activity in clinical situations.

Acknowledgments

This research was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports and Culture, Japan (S.O., 00223884). Sumitomo Seiyaku Co. (Osaka, Japan) generously supplied IFN-α (Sumiferon). We are grateful to them.

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