Relationship Between 24-Hour Rhythm in Antiviral Effect of Interferon-\(\beta\) and Interferon-\(\alpha/\beta\) Receptor Expression in Mice

Hiroshi Takane\(^1,2\), Shigehiro Ohdo\(^1,*\), Reiji Baba\(^1\), Satoru Koyanagi\(^3\), Eiji Yukawa\(^1\) and Shun Higuchi\(^1\)

\(^1\)Clinical Pharmacokinetics, Division of Clinical Pharmacy, Department of Medico-Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan
\(^2\)Department of Hospital Pharmacy, Faculty of Medicine, Tottori University, 36-1, Nishi-Machi, Yonago 683-8504, Japan
\(^3\)Department of Biochemistry, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1, Nanakuma, Jonan-Ku, Fukuoka 814-0180, Japan

Received May 17, 2002 Accepted August 27, 2002

ABSTRACT—The influence of interferon-\(\beta\) (IFN-\(\beta\)) dosing time on antiviral activity was investigated in ICR male mice under light-dark cycle conditions (lights on at 07:00, off at 19:00) with food and water available ad libitum. There was a significant dosing time-dependent change in 2',5'-oligoadenylate synthetase (2',5'-OAS) activities, as an index of antiviral activity, in liver at 12 h after IFN-\(\beta\) (15 MIU/kg, i.v.) injection. IFN-\(\beta\)-induced 2',5'-OAS activity was more potent after the drug injection during the late dark phase. The higher antiviral effect of IFN-\(\beta\) was observed when the interferon-\(\alpha/\beta\) receptor (IFNAR) expression in the liver increased, and the lower effect was observed when its expression decreased. IFN-\(\beta\)-induced fever was more serious after IFN-\(\beta\) injection from the late dark phase to the early light phase. A significant dosing time-dependent change was demonstrated for plasma IFN-\(\beta\) concentrations, which showed a higher level during the light phase and a lower level during the dark phase. The dosing time-dependent change of plasma IFN-\(\beta\) concentrations was not associated with that of the antiviral effect or fever induced by IFN-\(\beta\). These results suggest that selecting the most suitable dosing time of IFN-\(\beta\), associated with the 24-h rhythm of IFNAR expression in the liver, may be important to increase effectively the antiviral activity of the drug in experimental and clinical situations.

**Keywords**: Interferon, Interferon receptor, 24-Hour rhythm, Chronopharmacology, Chronopharmacokinetics

A 24-h rhythm has been demonstrated for a large number of physiological functions (1 – 3). The circadian oscillator in mammals is located in the suprachiasmatic nuclei (SCN) of the hypothalamus and plays a critical role in adapting endogenous physiological functions to cyclic environmental factors such as light, temperature and social communication. On the other hand, responses to a variety of drugs show 24-h rhythmicity (4 – 6). Use of a chronopharmacological strategy can improve the tumor response to treatment, and overall survival rates and reduce drug toxicities in humans (7, 8). The mechanisms involved in the 24-h rhythm of drug susceptibility have been examined from the viewpoints of the sensitivity of living organisms to drugs and/or the pharmacokinetics of drugs.

Interferons (IFNs) are multifunctional cytokines that have not only antiproliferative and immunological effects but also potent antiviral effects (9). IFNs have been widely used to treat patients with various types of cancer and hepatitis. On the other hand, adverse effects such as fever, headache and leukopenia are frequently observed in patients treated with IFNs (9). In particular, fever is a frequent side effect in patients with IFN therapy. One approach to increase the efficiency of IFNs treatment is the administration of the drugs at the time that they are most effective and/or tolerated. Certainly, the fever (5, 10) or antiviral activity (10) induced by IFN-\(\alpha\) is significantly affected by dosing time. However, the exact mechanisms have not been clarified yet.

IFN elicits biological activity through binding to specific receptors (11). IFN-\(\alpha\) and -\(\beta\) bind equally to interferon-\(\alpha/\beta\) receptor (IFNAR) (12). IFN-\(\alpha\) and -\(\beta\) are the only effective antiviral agent that eliminates hepatitis virus from hepatocytes. However, only about half of the patients with hepatitis C virus (HCV) infection receiving IFN-\(\alpha\) therapy are able to eliminate virus or normalize serum aminotrans-
ferrase (13, 14). The response to IFN-α therapy is associated with the amount of serum HCV-RNA (15) and HCV genotype (16). Furthermore, the expression of IFNAR in the liver is significantly related to the response to IFN therapy (17, 18). Therefore, the inter or intraindividual variability of IFNAR level may be an important host factor influencing the response to IFN-α and -β.

Previous studies have demonstrated that the opiate receptor binding has a significant 24-h rhythm with a higher level during the dark phase (19). The higher analgesic effectiveness of morphine is observed after drug injection during the dark phase in mice (20). Also, the interindividual variability in response to a β-adrenoreceptor agonist or antagonist can be explained by the different β-adrenoreceptor density on lymphocytes (21, 22). Thus, the rhythmic change of receptor expression may mainly contribute to that of the effect induced by a receptor-mediated drug. However, little is known about the relationship between the 24-h rhythm of the pharmacological effect induced by receptor-mediated drugs and their receptor expression on target tissues.

This study was designed to clarify the influence of IFN-β dosing time on antiviral activity, 2',5'-oligoadenylate synthetase (2',5'-OAS) mRNA level, and activity in mice. The mechanism underlying the dosing time-dependent action of IFN-β was investigated from the viewpoints of the 24-h rhythm of IFNAR mRNA expression in liver and IFN-β pharmacokinetics. The relationship between the 24-h rhythm of the pharmacological effect induced by IFN-β and IFNAR expression was clarified.

MATERIALS AND METHODS

Animals

Male ICR mice (5-week-old) were purchased from the Charles River Japan, Inc. (Kanagawa). They were housed 8 – 10 per cage under standardized light-dark cycle conditions (lights on at 07:00, off at 19:00) at 24 ± 1°C and 60 ± 10% humidity with food and water available ad libitum.

Drugs

The lyophilized powder of natural human IFN-β (Feron®) (Toray Industries, Inc., Tokyo) was dissolved in saline and intravenously (i.v.) injected (0.05 ml/kg of body weight).

Experimental design

To investigate the influence of IFN-β dosing time on 2',5'-OAS mRNA level and activity, groups of 7 – 8 mice were injected with IFN-β (15 MIU/kg, i.v.) or saline at one of the following times of day: 09:00, 13:00, 17:00, 21:00, 01:00 or 05:00. The liver samples were quickly removed at 4 h (for mRNA) or 12 h (for activity) after IFN-β (15 × 10⁶ IU [15 MIU]/kg, i.v.) or saline injection and placed into ice-cold tubes. To examine the influence of IFN-β dosing time on rectal temperature, groups of 6 mice were injected with IFN-β (15 MIU/kg, i.v.) or saline at each of the six times outlined above. Rectal temperatures were measured at 0.5 h after IFN-β or saline injection. To investigate the influence of IFN-β dosing time on cyclooxygenase (COX)-1 or -2 mRNA expression in the hypothalamus, groups of 5 mice were injected with IFN-β (15 MIU/kg, i.v.) or saline at 09:00 or 21:00. The hypothalamus was removed at 0.5 h after IFN-β or saline injection. In order to study the 24-h rhythm of IFNAR mRNA expression in the liver or lymphocytes, each of tissues was removed from groups of 5 – 6 mice at each of the six times outlined above. To investigate the time-dependent change in specific binding of IFN-α to IFNAR on lymphocytes, lymphocytes were obtained from groups of 5 mice at 17:00 or 05:00. To examine the 24-h rhythm of plasma IFN-β concentration at 2.5 h after IFN-β injection, groups of 5 – 6 mice were injected with IFN-β (15 MIU/kg, i.v.) or saline at each of the six times outlined above. Also, to determine the influence of IFN-β dosing time on the time course of plasma IFN-β concentrations, groups of 5 – 6 mice were given an intravenous injection of IFN-β (15 MIU/kg) at 09:00 or 21:00. Blood samples were collected at 0.167, 0.5, 1, 1.5 or 3 h after IFN-β injection.

RNA extraction and RT-PCR

Total RNA was extracted from liver or lymphocytes by using TRIZOL® solution (BRL, Bethesda, MD, USA). The reverse transcription of RNA and cDNA amplification were performed with a one-step RT-PCR system (BRL). RT-PCR reactions were performed with 2',5'-OAS, COX-1, COX-2, IFNAR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers. The following primers used: 5'-GCAAGCCTGTACCCAGAATCT-3' and 5'-TAGCCACACATCAAGCCTTCTTCA-3' for 2',5'-OAS (GenBank accession number: X04958), 5'-AGGAGATGCTGCTGAGTTGG-3' and 5'-AATCTGTATTTCTGAGTGGCC-3' for COX-1 (GenBank accession number: M34141), 5'-ACACTCTATCACCTGGCACCC-3' and 5'-TTCAGGGAGAAGCCGTTTGGC-3' for COX-2 (GenBank accession number: M82866), 5'-CATGGCGTGCTATATTGTTCC-3' and 5'-ATGCGTTGTTAAAGGTGTAC-3' for IFNAR (GenBank accession number: U06237), 5'-GACCTCAACTATGGTCTACA-3' and 5'-ACTCCAGCAGACATCTACGAC-3' for GAPDH (GenBank accession number: M32599). The PCR product was electrophoresed through a 3% agarose gel including 0.2 μg/ml ethidium bromide, and the gel was photographed under UV light (302 nm). The band intensity was quantified using a NIH image analysis program and normalized against GAPDH.

Determination of 2',5'-OAS activity

Liver was perfused with phosphate-buffered saline.
albumin, 0.1% sodium azide, 10 /g 10^9 2.5 mM CaCl
RPMI 1640 medium containing 0.25% bovine serum
isolated as described above and resuspended in ice-cold
Inc., Irvine, CA, USA). The splenic lymphocytes were
isolated by a density-gradient separation
Lymphocyte isolation

Specific IFN-α binding assay

The iodination of IFN-α reduces its biological potency by <30% (25). Both IFN-α and -β cross-compete for the
same receptor (12). Therefore, recombinant human IFN-α
(Pepro Tech EC, Ltd., London, UK) was used as ligand to
the specific receptor of IFN-β. IFN-α was iodinated using
a solid-phase lactoperoxidase kit (ICN Pharmaceuticals,
Inc., Irvine, CA, USA). The splenic lymphocytes were
isolated as described above and resuspended in ice-cold
RPMI 1640 medium containing 0.25% bovine serum albumin, 0.1% sodium azide, 10 μg/ml protamine sulfate,
2.5 mM CaCl₂, and the indicated concentrations of [125I]-
IFN-α. The binding assay was performed at 4°C for 2 h
with a reaction mixture (a total volume 200 μl) containing
various concentrations of [125I]-IFN-α and 1 x 10^6 viable
cells. After incubation, the reaction mixture was layered
over 200 μl of heated-inactivated fetal bovine serum and
centrifuged at 7,000 x g for 1 min. The supernatant was
removed. Thereafter, the tube tip containing bound ligand
was amputated and the radioactivity was measured using
a gammar counter (ARC-360; Aloka Co., Mitaka). Non-
specific binding was evaluated in the presence of at least
250-fold excess of unlabeled IFN-α. Specific binding was
defined as nonspecific binding subtracted from the total
binding. The data were plotted according to the method of
Scatchard (26). A molecular weight of 20,000 was assumed
for the calculation of the receptor number per cell and the
dissociation constant (Kd).

Determination of plasma IFN-β concentrations

Blood samples were collected by orbital sinus and
plasma samples were obtained after centrifugation at
600 x g for 3 min (Kubota Hematocrit KH-120A; Kubota,
Tokyo) and stored at ~20°C until assayed. Plasma IFN-β
concentrations were determined by the enzyme-linked-
imunosorbent assay method (Human IFN-β ELISA kit,
Toray Co.).

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by the non-
linear least-squares method, following the two-compartment
model: total body clearance (CL), central volume of
distribution (Vc), distribution rate constant from central to
peripheral compartment (k12) and distribution rate constant
from peripheral to central compartment (k21).

Statistical analyses

Analysis of variance (ANOVA) and Tukey’s test were
applied for the multiple comparison. Student’s t-test was
used for two independent groups. The 5% level of prob-
ability was considered to be significant.

RESULTS

Influence of IFN-β dosing time on liver 2',5'-OAS mRNA
expression and activity

The 2',5'-OAS mRNA level in liver at 4 h after IFN-β
(15 MIU/kg, i.v.) injection was significantly higher in mice
treated with the drug at 01:00 or 05:00 (P<0.01, respec-
tively, Fig. 1). However, the 2',5'-OAS mRNA in liver was
not detected at 4 h after saline injection at any dosing times.
On the other hand, the 2',5'-OAS activity in liver at 12 h
after IFN-β injection at 05:00 or 17:00 was significantly
higher when compared with that after saline injection at the
corresponding dosing time (P<0.01, respectively, Fig. 2).
Furthermore, the 2',5'-OAS activity in liver at 12 h after
IFN-β injection at 05:00 was significantly higher than that
after IFN-β injection at 17:00 (P<0.05). No dosing time-
dependent change was observed for 2',5'-OAS activity in
liver at 12 h after saline injection.
Influence of IFN-β dosing time on rectal temperature

The rectal temperature showed a significant 24-h rhythm with a lower level during the light phase and a higher level during the dark phase (P<0.01, ANOVA, Fig. 3). The percent change of rectal temperature at 0.5 h after IFN-β (15 MIU/kg, i.v.) injection at 09:00, 13:00 or 05:00 was significantly higher than that after saline injection at the corresponding dosing time (09:00: P<0.01, 21:00: P<0.05, Fig. 5). Furthermore, it was significantly higher in mice injected with IFN-β at 09:00 than at 21:00 (P<0.05). However, the COX-1 mRNA was not induced by IFN-β injection at 09:00 or 21:00 (Fig. 5).

24-Hour rhythm of IFNAR expression in liver and lymphocyte

The IFNAR mRNA level in liver and lymphocyte showed a significant 24-h rhythm (P<0.01, respectively, ANOVA, Fig. 6). The IFNAR mRNA level in liver was higher during the late dark phase and lower during the late light phase. The IFNAR mRNA level in lymphocytes was higher during the late dark phase and the early light phase, and lower during the late light phase and the early dark
phase. Furthermore, the number of IFNAR per cell of lymphocytes, calculated from the intercept of the Scatchard plot on the abscissa, was significantly larger in cells prepared at 05:00 than at 17:00 ($P<0.01$, Table 1). The apparent $K_d$ showed no significant difference between cells prepared at 05:00 and 17:00.

**Influence of dosing time on IFN-β pharmacokinetics**

The plasma IFN-β concentrations at 2.5 h after IFN-β (15 MIU/kg, i.v.) injection showed a significant 24-h rhythm with higher levels during the light phase and lower levels during the dark phase ($P<0.01$, ANOVA, Fig. 7). The influence of dosing time on the time course of plasma IFN-β concentrations after IFN-β injection is shown in Table 2. The time course of plasma IFN-β concentrations after IFN-β injection decreased in a biexponential fashion. Plasma IFN-β concentrations at 1.5 and 3 h after IFN-β injection at 09:00 were significantly higher when compared with those after the drug injection at 21:00 ($P<0.05$, respectively). Table 3 shows the pharmacokinetic parameters after IFN-β injection. CL was significantly higher in mice injected with IFN-β at 21:00 than at 09:00 ($P<0.05$).

**DISCUSSION**

Antiviral efficacy of IFN is mediated through production of 2',5'-OAS activated by double-stranded RNA (27). 2',5'-OAS activates a latent ribonuclease, can cleave single-stranded RNA, and impairs replication of various virus (28, 29). In this study, the 2',5'-OAS mRNA induction and its activity in liver after IFN-β injection were significantly more potent in mice injected with the drug during the late dark phase. This result was supported by a previous chronopharmacological finding of IFN-α (10).

IFN elicits antiviral activity by binding to the specific
receptor on the cell surface (30). In IFNAR-deficient cell lines, IFN-α and -β fail to induce 2',5'-OAS activity and protect against viral infection (31). Furthermore, the level of IFNAR expression in liver is closely related to the efficacy of IFN-α therapy in patients with chronic hepatitis C (18, 32). In the present study, the level of IFNAR mRNA in liver showed a significant 24-h rhythm with a higher level during the late dark phase and lower level during the late light phase. In addition, we examined the binding assay for IFNAR by using lymphocytes because the homogenization and processing of membranes for binding alter the binding kinetics of the receptor (33). The number of IFNAR per cell was significantly larger in cells obtained at 05:00 than at 17:00. These results indicate that the rhythmic change of IFNAR mRNA level is associated with that of the number of IFNAR. Moreover, the 24-h rhythm of IFNAR mRNA level in the liver was consistent with the dosing time-dependent change of 2',5'-OAS mRNA induction and its activation in liver by IFN-β. On the other hand, the plasma IFN-β concentrations at 2.5 h after IFN-β injection was higher during the light phase and lower during the dark phase. Namely, the rhythmicity of plasma IFN-β concentration was out of phase with the dosing time-dependent change of 2',5'-OAS activation induced by IFN-β. Therefore, the time-dependency in 2',5'-OAS activation of IFN-β seems to be due to not the rhythmicity of IFN-β pharmacokinetics but that of IFNAR expression.

The change of rectal temperature at 0.5 h after IFN-β injection during the late dark phase and the early light phase was significantly higher than that after saline injection at the corresponding dosing time. However, no significant dosing time-dependence was observed for IFN-β concentrations at 0.5 h after IFN-β injection. IFN-α-induced fever

| Table 1. Time-dependent difference of IFNAR expression on lymphocytes prepared at 17:00 or 05:00 |
|-------------------------------------------------|-------------------------------------------------|
| IFNAR                                           | Time of cell preparation (clock hours)           | Student’s t-test |
|                                                 | 17:00                                           | 05:00           |       |
| Number (sites/cell)                             | 218 ± 22                                        | 341 ± 29        | P<0.01|
| Apparent Kd (× 10^{-10} M)                      | 4.02 ± 0.39                                     | 4.43 ± 0.29     | N.S.  |

Each value is the mean with S.E.M. of 5 mice.
is mediated by prostaglandin E₂ (PGE₂) production and/or an opioid receptor mechanism in the hypothalamus (34). The dosing time-dependent difference of fever induced by IFN-α is caused by that of PGE₂ production induced by the drug (10). COX-2 plays a major role in the rapid production of PGE₂ and fever induced by IL-1β (35). The hypothalamic COX-2 mRNA level, but not COX-1 mRNA level, was significantly higher in mice injected with IFN-β at 09:00 than at 21:00. Glucocorticoid inhibits the elevation of COX-2 mRNA as well as PGE₂ formation induced by IL-1β (36, 37). In rodents, plasma glucocorticoid hormone such as corticosterone shows 24-h rhythmicity with elevation during the late light phase and early dark phase. PGE₂ concentration in cerebrospinal fluid shows 24-h rhythm with higher level during the light phase, but with less magnitude in rats (38). In this study, the serious fever induced by IFN-β was observed when plasma glucocorticoid level was lower (data not shown). Thus, the time-dependency in fever induced IFN-β may be due to the rhythmicity of plasma glucocorticoid level.

A significant dosing time-dependent difference was also demonstrated for the pharmacokinetic parameter of IFN-β, which showed higher CL for injection at 21:00 than for injection at 09:00. The rhythmicity of CL seems to contribute to that of plasma IFN-β concentrations. The predominant pathway of IFN-β elimination is the liver (39). IFN-α is internalized via receptor-mediated endocytosis and catabolized intracellularly by lysosomal proteinases in metabolic tissue (40). The receptor-mediated saturable clearance of granulocyte colony-stimulating factor mainly contributes to total clearance at lower doses (41). However, CL was significantly higher in mice injected with IFN-β at 21:00 than at 09:00, although the IFNAR mRNA level in liver was higher at 09:00 than at 21:00. Thus, the rhythmicity of IFNAR expression in liver may not contribute to the dosing time-dependent change of IFN-β pharmacokinetics.

In conclusion, we have shown here the dosing time-dependency of the antiviral effect induced by IFN-β associated with that of IFNAR expression in the liver. The fever induced by IFN-β also showed a significant dosing time-dependent change. Furthermore, the rhythmic change of IFN-β pharmacokinetics failed to elucidate that of pharmacological effects induced by IFN-β, although there was a significant dosing time-dependent difference in plasma IFN-β concentrations. Previous studies have shown that the interindividual variability in response of β-adrenoceptor agonist or antagonist can be related to that in β-adrenoceptor density on lymphocytes (21, 22). Therefore, the choice of dosing time associated with the rhythmicity of IFNAR expression may help to achieve a rational chronopharmacological strategy for increasing the therapeutic effects of IFN-β. Also, the 24-h rhythm of IFNAR level on lymphocytes may be a reference marker for that of IFNAR level in the liver, since IFNAR mRNA levels in both

### Table 2. Influence of dosing time on plasma IFN-β concentrations after IFN-β (15 MIU/kg, i.v.) injection at 09:00 or 21:00

<table>
<thead>
<tr>
<th>Time after drug injection (h)</th>
<th>Plasma IFN-β concentration (kIU/ml)</th>
<th>Time of drug injection (clock hours)</th>
<th>Student’s t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>09:00</td>
<td>21:00</td>
</tr>
<tr>
<td>0.167</td>
<td>59.014 ± 3.715</td>
<td>52.790 ± 3.980</td>
<td>N.S.</td>
</tr>
<tr>
<td>0.5</td>
<td>14.121 ± 0.674</td>
<td>12.906 ± 0.773</td>
<td>N.S.</td>
</tr>
<tr>
<td>1.0</td>
<td>6.048 ± 0.365</td>
<td>5.005 ± 0.371</td>
<td>N.S.</td>
</tr>
<tr>
<td>1.5</td>
<td>3.450 ± 0.241</td>
<td>2.688 ± 0.225</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>3.0</td>
<td>1.172 ± 0.113</td>
<td>0.789 ± 0.052</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Each value is the mean with S.E.M. of 5 – 6 mice.

### Table 3. Influence of dosing time on pharmacokinetic parameters after IFN-β (15 MIU/kg, i.v.) injection at 09:00 or 21:00

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Time of drug injection (clock hours)</th>
<th>Student’s t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>09:00</td>
<td>21:00</td>
</tr>
<tr>
<td>CL (l/h per kg)</td>
<td>0.414 ± 0.012</td>
<td>0.481 ± 0.028</td>
</tr>
<tr>
<td>Vc (l/kg)</td>
<td>0.106 ± 0.006</td>
<td>0.128 ± 0.547</td>
</tr>
<tr>
<td>k12 (l/h)</td>
<td>1.783 ± 0.237</td>
<td>1.393 ± 0.124</td>
</tr>
<tr>
<td>k21 (l/h)</td>
<td>1.199 ± 0.076</td>
<td>1.232 ± 0.041</td>
</tr>
</tbody>
</table>

Each value is the mean with S.E.M. of 5 – 6 mice.
lymphocytes and liver exhibit similar rhythmicities. However, it is still unclear which factors control the rhythmicity of IFNAR expression. To clarify the mechanisms may lead to the discovery of a more convenient rhythmic marker for IFNAR expression in each tissue. From this question, we might go on to a detailed examination of its mechanisms.

Acknowledgments

We are indebted to Toray Industries, Inc. (Tokyo) for providing IFN-β (Interferon-β, Feron®) used in this study. This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas “Cancer” (S.O., 14030062); a Grant-in-Aid for Scientific Research (C) (S.O.,13672391) and a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; a Grant-in-Aid from the Pharmacological Research Foundation, Tokyo (S.O.); and a Grant-in-Aid from Japan Research Foundation for Clinical Pharmacology (S.O.).

REFERENCES


4 Ohdo S, Koyanagi S, Suyama H, Higuchi S and Aramaki H: Circadian rhythm of liver exhibit similar rhythmicities. How they go on to a detailed examination of its mechanisms.

IFN-β expression in each tissue. From this question, we might go on to a detailed examination of its mechanisms.

Acknowledgments

We are indebted to Toray Industries, Inc. (Tokyo) for providing IFN-β (Interferon-β, Feron®) used in this study. This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas “Cancer” (S.O., 14030062); a Grant-in-Aid for Scientific Research (C) (S.O.,13672391) and a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; a Grant-in-Aid from the Pharmacological Research Foundation, Tokyo (S.O.); and a Grant-in-Aid from Japan Research Foundation for Clinical Pharmacology (S.O.).


4 Ohdo S, Koyanagi S, Suyama H, Higuchi S and Aramaki H: Circadian rhythm of liver exhibit similar rhythmicities. How they go on to a detailed examination of its mechanisms.

Acknowledgments

We are indebted to Toray Industries, Inc. (Tokyo) for providing IFN-β (Interferon-β, Feron®) used in this study. This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas “Cancer” (S.O., 14030062); a Grant-in-Aid for Scientific Research (C) (S.O.,13672391) and a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; a Grant-in-Aid from the Pharmacological Research Foundation, Tokyo (S.O.); and a Grant-in-Aid from Japan Research Foundation for Clinical Pharmacology (S.O.).
to interferons $\alpha$ and $\beta$ and alters macrophage responses. Proc Natl Acad Sci USA 92, 11284 – 11288 (1995)


33 Davis ME, Akera T and Brody TM: Reduction of opiate binding to brain stem slices associated with the development of tolerance to morphine in rats. J Pharmacol Exp Ther 211, 112 – 119 (1979)


