Metallothionein Synthesis Induced by Interferon α/β in Mice of Various Zinc Status

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Institute of Biomedical Sciences, Fukushima Medical College, Fukushima 960-12, *Department of Biochemical Toxicology, School of Pharmaceutical Sciences, Showa University, Tokyo 142, †Taiho Pharmaceutical Co., Ltd., Tokushima 770, and ‡Japan Immunoresearch Laboratories Co., Ltd., Takasaki 370

Sato, M., Yamaki, J., Oguro, T., Yoshida, T., Nomura, N. and Nakajima, K. Metallothionein Synthesis Induced by Interferon α/β in Mice of Various Zinc Status. Tohoku J. Exp. Med., 1996, 178 (3), 241–250 — We studied the ability of interferon α/β (IFN) to induce metallothionein (MT) synthesis in mice. Male mice were injected intraperitoneally with mouse IFN (5 × 10⁴ IU/mouse). Plasma Zn levels were reduced at 4 hr after injection, reached a minimum value at 6 hr, and then returned to the control level at 8 hr. Hepatic MT concentrations began to increase at 4 hr and reached maximum values at 6 hr. Induction of MT gene expression and protein synthesis was confirmed by Northern blot analysis and radioimmunoassay, respectively. The induction of MT synthesis in the liver by IFN was dose-dependent. The data suggest that induction of MT-mRNA and the protein in the liver by IFN occurs rapidly but is rather transient. Furthermore, MT synthesis was not induced by IFN in the liver of mice given a Zn-deficient diet, whereas IFN induced increases in the activity of 2', 5'-oligoadenylate synthetase in the spleen were unaffected by Zn status. Thus, induction of hepatic MT synthesis by IFN is influenced by Zn status. —— interferon (IFN); metallothionein (MT); 2', 5'-oligoadenylate synthetase (2', 5'-AS); zinc deficiency

Metallothionein (MT) is a cysteine-rich, low molecular weight (6,000-7,000) metal-binding protein which is found in most tissues (Kagi and Kojima 1987). MT is induced in many organs not only by heavy metals such as zinc (Zn), copper (Cu), cadmium (Cd) and mercury (Hg), but also by hormones and cytokines (Sato and Bremner 1993). MT is believed to detoxify heavy metals, to regulate the metabolism of the essential metals, and may also act as a radical scavenger. The latter role is based on the findings that MT synthesis is induced by a variety of

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physical and chemical stresses including oxidative stress (Sato and Bremner 1993) and that MT reacts with hydroxyl radicals in vitro with a high rate constant (Thornalley and Vasak 1985).

We have previously reported that MT synthesis by oxidative stress is associated with cytokines (Sato et al. 1995). In fact, interleukin-1 (IL-1) (Cousins and Leinart 1988), tumor necrosis factor-α (TNF) and IL-6 rapidly induce MT synthesis in the liver, heart and lung of rats (Sato et al. 1994). Induction of MT-mRNA by interferon (IFN) has also been reported (Friedman and Stark 1985), but there is little information on induction of the protein.

Synthesis of IFN α and β is induced by many inflammatory stimuli and by agents such as viruses, double stranded RNA, microorganisms and protozoa. In the present paper we describe the ability of natural IFN to induce MT synthesis in mice, and the dependence of the response on the Zn status of the animals.

Materials and Methods

Chemicals

Bolton-Hunter reagent was obtained from ICN Biochemicals (Irvine, CA, USA), and donkey anti-sheep IgG serum was obtained from the Scottish Antibody Production Unit (Carluke, Scotland, UK). Anti rat liver MT-I sheep serum was kindly provided by Dr. Ian Bremner, Rowett Research Institute, Aberdeen, Scotland, UK. Mouse natural IFNα/β (2.80×10⁷ IU/mg protein) and human natural IFNα (2.0×10⁸ IU/mg protein) were provided by Dr. Kiyoshi Ishi, Otsuka Pharmaceutical Co. Ltd., Tokushima. All other chemicals were of the highest grade commercially available.

Animals and treatment

Experiment 1. Male ddY mice (7 weeks old, Funabashi Farm Co., Funabashi), weighing approximately 25 g were housed in groups of five per cage in an environmentally controlled room (light on 0700–1900; temperature 23±1.5°C; humidity 55.5%). Animals were allowed free access to water and commercial chow diet (Clea Japan Inc., Tokyo) ad libitum. Mice were injected intraperitoneally (i.p.) with mouse IFN α/β (5×10⁶ IU/mouse). Under pentobarbital anesthesia, blood was collected by cardiac puncture into a heparinized syringe, and centrifuged in a microtube to separate plasma from red cells. The mice were sacrificed 4, 6, 8 and 16 hr after the injection. Tissues were quickly removed and stored at −80°C before analyses. To compare the ability of mouse IFN and human IFNα to induce MT synthesis, another group of mice was injected i.p. with human IFNα (5×10⁶ IU/mouse) and sacrificed 6 hr after injection.

Experiment 2. Male C3H/HeJ mice (4 week old), weighing about 18–20 g were used. Zn-deficient mice were produced by feeding animals with a semi-synthetic diet (Oriental Yeast Co. Ltd., Tokyo) containing <1 mg Zn/kg for 2 weeks. Control mice received a semi-synthetic diet containing 55.2 mg Zn/kg
Tissue preparation and analysis

Tissues were homogenized in cold 50 mM Tris-HCl buffer (pH 7.4), to make a 10% homogenate (w/v). Portions of each homogenate were digested with acid mixture (HNO₃/HClO₄/H₂SO₄, 5:2:1 vol/vol or HNO₃/HClO₄, 5:1). Tissue homogenates were digested, and diluted with ultrapure water (Japan Millipore Ltd., Tokyo), whereupon they were analyzed for Zn and Cu by atomic absorption spectrometry (Z-6100; Hitachi Ltd., Tokyo). The Zn and Cu concentrations in the plasma were directly measured by atomic absorption spectrometry after dilution with ultrapure water.

Other portions of the homogenates were centrifuged at 1,500 × g for 10 min before measuring MT concentrations in the supernatants by the Cd-heme method (Onosaka et al. 1978; Onosaka and Cherian 1981) or by radioimmunooassay (Mehra and Bremner 1983). Total RNA was isolated from the rat liver using the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi 1987). After electrophoresis, Northern blot analysis was used to measure MT-mRNA with a mouse MT-I cDNA probe (kindly donated by Drs. Imura and Naganuma, Kitasato University, Tokyo) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA with a cDNA probe to human GAPDH (kindly donated by Dr. K. Nose, Showa University, Tokyo). Probes were labeled with ³²P by the multiprimer method (Feinberg and Volgelstein 1983). For quantitative analysis, the radioactivity of bands revealed on an imaging plate, was measured in a Fuji Bioimage analyzer BAS 3000 J (Fuji Photo Film Co., Tokyo). Activities of 2',5'-oligoadenylate synthetase (2',5'-AS) were measured by radioimmunoassay (Sawai et al. 1984) using commercially available 2-5A kit (Eiken Chemical Co., Tokyo).

Statistical analysis

Statistical comparisons were performed using Student’s t-test. The acceptable level of significance was set at p < 0.05.

Results

Experiment 1. Fig. 1 demonstrates the time course of the changes in plasma concentrations of Zn and Cu following administration of mouse IFNα/β. Plasma Zn levels rapidly decreased at 4 hr and reached a minimum at 6 hr. Then, the Zn concentration returned to the control levels of 0 hr and finally exceeded the control levels at 16 hr. In contrast, the concentration of Cu did not change during the experimental period.

The concentration of MT in the liver, as determined by the Cd-heme method,
Fig. 1. Time course for plasma concentrations of Zn and Cu following interferonα/β (IFNα/β) administration. Male mice were injected i.p. with mouse IFN (5 × 10⁶ IU). Mice were killed at 0, 4, 6, 8 and 16 hr after injection. Values are mean ± s.e. of 5–8 mice. *Significant difference from control (0 hr) at p < 0.05.

significantly increased after injection, reached a maximum at 6 hr, and then decreased at 8 hr (Fig. 2). Thus, there was a close connection between the induction of MT synthesis in the liver and the rapid decrease in plasma Zn levels, and the high level of MT synthesis was observed at early time after injection. The rapid induction in hepatic MT synthesis by IFNα/β was confirmed by determination of MT-mRNA by Northern blot analyses (Fig. 3). Normalization of the MT-mRNA levels to those levels for GAPDH-mRNA indicated that hepatic MT-mRNA levels in the IFN-treated mice after 6 hr was 3 times greater than in control animals. The maximum induction of MT-mRNA was observed at 6 hr (data not shown).

No increase was found in MT concentrations in kidneys from IFN-treated...

Fig. 2. Changes in MT synthesis in the liver following IFNα/β administration. Each point shows the mean ± s.e. of 5–8 mice.
mice (data not shown).

Experiment 2. As was expected, the plasma concentrations of Zn were reduced by about 50% in mice given the Zn-deficient diet for 2 weeks (Fig. 4). Administration of IFNα/β did not cause any changes in plasma concentration of Zn in mice given Zn-adequate or Zn-deficient diet. Since IFNα/β was injected into mice twice, this may be due to a rebound effect at 20 hr after the first injection, as shown in Fig. 1. There was also a decrease in the hepatic concentra-

Fig. 4. Plasma concentrations of Zn and Cu in mice given Zn-adequate or Zn-deficient diet. Male mice were injected i.p. with IFNα/β (IFN1: 2.5 × 10^6, IFN2: 5 × 10^6 IU) at 20 and 6 hr before sacrifice. *Significant difference from saline-treated control (Sal) at p < 0.05.
tion of Zn in mice given the Zn-deficient diet (29.0 ± 0.2 μg/g, mean ± s.e. of 5 mice) compared with the mice given the Zn-adequate diet (31.0 ± 0.6 μg/g, n = 5, p < 0.05).

Injection of mice with IFNα/β significantly increased the hepatic concentration of MT determined by Cd-heme method in mice given the Zn-adequate diet (Fig. 5). The increase was dose-dependent. However, in mice given the Zn-deficient diet, the MT levels did not change at either dose of IFN. Induction of MT protein synthesis was confirmed by radioimmunoassay using MT-I specific antibody. The hepatic concentration of MT-I increased in a dose-dependent manner in mice given the Zn-adequate diet (Control, 1.4 ± 0.3 μg/g; IFN1, 4.2 ±

![MT concentration graph](image1)

**Fig. 5.** Hepatic concentrations of MT following IFNα/β administration in mice given Zn-adequate or Zn-deficient diet. Male mice were injected i.p. with IFNα/β (IFN1: 2.5 × 10⁵, IFN2: 5 × 10⁵ IU) at 20 and 6 hr before sacrifice. Values are mean ± s.e. of 5 mice. *Significant difference from saline-treated (Sal) at p < 0.05.

![2',5'-AS activity graph](image2)

**Fig. 6.** Effect of IFNα/β administration on 2', 5'-oligoadenylate synthetase (2', 5'-As) activities in the spleen of mice given Zn-adequate or Zn-deficient diet. Male mice were injected i.p. with IFNα/β (IFN1: 2.5 × 10⁵, IFN2: 5 × 10⁵ IU) at 20 and 6 hr before sacrifice. Values are the mean ± s.e. of 5 mice. *Significant difference from saline-treated control (Sal) at p < 0.05.
Fig. 7. Concentrations of Zn and Cu in the plasma, and MT in the liver and kidney following human IFNα administration in mice. Male mice were injected i.p. with human IFNα (5 × 10^5 IU) and sacrificed at 6 hr after injection. Values are mean ± s.e. of 3–5 mice.

0.5; IFN2, 7.5 ± 1.4; Mean ± s.e. n = 5), whereas the MT-I concentration did not increase in mice given the Zn-deficient diet (Control, 0.3 ± 0.1 μg/g; IFN1, 0.4 ± 0.1; IFN2, 0.4 ± 0.1; Mean ± s.e. n = 5). On the other hand, 2′, 5′-AS activities were significantly increased by IFNα/β administration in the spleens of mice given either Zn-adequate or Zn-deficient diet (Fig. 6). The extent of the increase was similar between the two dose groups, suggesting that deficiency did not impair the ability to respond to IFN.

Administration of human IFNα did not cause any changes in the concentrations of Zn and Cu in plasma, or MT in tissues (Fig. 7), or of MT-mRNA in the liver (Fig. 3, lane right).

**DISCUSSION**

Although induction of mRNAs of several genes by IFN has been reported (Friedman and Stark 1985), synthesis of the gene products has not always been reported. Since turnover of some of the mRNAs induced by IFN is rapid, it is of interest whether the induced mRNAs are indeed translated, thereby producing functional proteins in response to treatment with IFN. Induction of MT-mRNA by IFNα (Friedman and Stark 1985) and IFNγ (Kusari et al. 1987; De et al. 1990) has been reported. The present study demonstrates that administration of mouse IFNα/β induced both expression of MT-mRNA and synthesis of MT protein, as determined by Northern blot analysis and by Cd-heme method or radioimmunoassay in mouse liver, respectively. Further, increased splenic activities of 2′, 5′-AS, a gene product known to be induced by IFN (Kusari et al. 1987), were observed.

IL-1, IL-6 and TNF induce MT synthesis in vitro and in vivo. Hepatic MT synthesis is induced rapidly after injection of IL-6 or TNF, with concentrations reaching a maximum at 6 hr, and remaining at high levels for 36 hr (Sato et al.
1994). In contrast, concentrations of hepatic MT after injection of IFNα/β declined rapidly after the maximum was attained at 6 hr. Thus, MT induction by IFNα/β occurred rapidly and was rather transient. In Chinese hamster ovary cells, induction of MT-mRNA by IFNα was also transient, apparently because of instability of the mRNA (Morris and Huang 1987).

In HeLa cells IFNγ induces MT-mRNA more effectively than IFNα, whereas IFNα induces 2', 5'-AS-mRNA more effectively than IFNγ in human rhabdomyosarcoma RD-114 cells (Kusari et al. 1987). The ability of IFNα to induce MT-II-mRNA is identical to that of IFNγ in RD-114 cells. Thus, induction of MT-mRNA seems to be specific for cells and IFN species. The induction ability of IFNβ has not previously been reported.

We have demonstrated that induction of MT synthesis by endotoxin is significantly reduced in the liver of rats given a Zn-deficient diet (Sato et al. 1984). Induction of MT synthesis by endotoxin is mediated through release of cytokines including IL-1, IL-6, and TNF (Liu et al. 1991). The present study has shown that induction of hepatic MT synthesis by one of these cytokines, viz. IFN, was dependent on Zn status, whereas IFN-induced increases in 2', 5'-AS activity in the spleen were unaffected by Zn status. Increased activity of 2', 5'-AS is found in the liver as well as spleen of IFN-treated mice, the activity in the liver being closely correlated with that in the spleen (Sugawara et al. 1988). Since IFNα induces 2', 5'-AS-mRNA (Kusari et al. 1987), it appears that in Zn deficiency IFN induces expression of some genes such as 2', 5'-AS, but not of others such as MT.

In some patients with advanced stages of hepatitis C, such as chronic hepatitis or liver cirrhosis, the virus seems to be resistant to the anti-virus activity of IFN, although the cause of this insensitivity is unknown (Arase et al. 1992). Under such conditions, the concentrations of Zn in the plasma (Arakawa et al. 1990) and liver (Boyett and Sullivan 1970; Kameda 1983) are significantly lower than those in healthy subjects. Zn deficiency could conceivably be one of the factors responsible for this insensitivity, because of its effect on protein synthesis. Further studies are required to establish the relationships between expression of gene products such as MT, zinc status and anti-virus activity of IFN.

The biological significance of the increase in MT levels in the liver in response to IFN is not fully known. However, we have demonstrated that the increase in liver MT induced by IL-6 or TNF can prevent the hepatotoxic effects of carbon tetrachloride, suggesting that MT has a role as a radical scavenger (Sato et al. 1995). Since MT induction by IFN seemed to be rather transient and mild, MT may play a role in a limited area in the cells. Further studies are required to elucidate the exact role of MT in the animal's response to IFN.

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References


