Inhibition by interferon ($\alpha + \beta$) of mouse liver regeneration and its reversal by putrescine

Shuhei Nishiguchi, Shuzo Otani, Isao Matsui-Yuasa, Seiji Morisawa, Takeyuki Monna*, Tetsuo Kuroki+, Kenzo Kobayashi+ and Sukeo Yamamoto+

*Departments of Biochemistry, *Public Health* and +*Third Department of Internal Medicine, Osaka City University Medical School, Asahimachi, Abeno-ku, Osaka 545, Japan*

Received 26 May 1986

Mouse interferon ($\alpha + \beta$) given to mice by intraperitoneal injection suppressed both the accumulation of putrescine and stimulation of DNA synthesis in liver caused by partial hepatectomy. The suppression of DNA synthesis was completely reversed by exogenous putrescine. The same results were obtained when core 2′,5′-oligoadenylate instead of interferon was given to partially hepatectomized mice. These results suggest that interferon inhibits putrescine formation through elevating the 2′,5′-oligoadenylate level and thus inhibits its DNA synthesis in the regenerating liver.

Interferon *2′,5′-Oligoadenylate* *Putrescine* *Partial hepatectomy* *Liver regeneration* *(Mouse)*

1. INTRODUCTION

Interferons (IFNs) are glycoproteins synthesized and secreted by a variety of cells in response to several classes of inducers, and exert their effects as a result of interaction with other cells [1]. Besides their effects on viral replication, cell motility and immunological process, IFNs have an antiproliferative effect on transformed and non-transformed cells [1,2], which suggests that IFN might be an antitumor agent. However, the mechanism of the inhibition of cell growth is not fully understood. Polyamines are important for cell growth, and ornithine decarboxylase, a rate-limiting enzyme of polyamine biosynthesis, is induced when resting cells are transformed to growing cells by various mitogens [3]. Sreevalsan et al. [4] and Sekar et al. [5] reported that IFN inhibits ornithine decarboxylase, suggesting close correlation between polyamine metabolism and the antiproliferative effect of IFN. However, there is no direct evidence that IFN inhibiting polyamine biosynthesis would inhibit cell growth. Here we show that IFN ($\alpha+\beta$) and core 2′,5′-oligoadenylate (core 2-5A) inhibit both the accumulation of putrescine and DNA synthesis in mouse regenerating liver and also that the inhibition of DNA synthesis is completely overcome by administration of putrescine. To our knowledge, this is the first report that putrescine blocks the antiproliferative effect of IFN and core 2-5A.

Abbreviations: IFN, interferon; 2-5A, 2′,5′-oligoadenylate; i.p., intraperitoneally

2. EXPERIMENTAL

2.1. *Materials*

IFN ($\alpha+\beta$) (5×10^6 IU/mg protein) produced in mouse L cells was kindly provided by the Basic Research Laboratories of Toray Industries, Kamakura. Core 2-5A was a product from P-L Biochemicals, Milwaukee, WI. Putrescine was purchased from Sigma, St. Louis, MO. [6-^{3}H]Thymidine (19.3 Ci/mmol) was from Du Pont/New England Nuclear, Boston, MA.

2.2. *Partial hepatectomy*

IFN ($\alpha+\beta$) dissolved in 0.9% NaCl solution was

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

injected into male mice (C_3H/HeN, aged 6 weeks) i.p. The mouse was then starved for 16 h, after which 70% of the liver was removed by the method of Higgins and Anderson [6]. Core 2-5A dissolved in 0.9% NaCl solution was injected i.p. immediately and again 2 h after the partial hepatectomy. Putrescrine (1 mg) dissolved in 0.2 ml of 0.9% NaCl solution was given i.p. three times, 8, 16, and 24 h after the operation.

2.3. *Measurement of [^{3}H]thymidine incorporation into DNA*

[^{3}H]Thymidine (10 μCi/100 g body wt) was injected into mice i.p. at various times after the partial hepatectomy. After 4 h of labelling with [^{3}H]thymidine, the mice were killed and the remaining liver was removed. The liver was homogenized with 4 vols of cold distilled water and then 1 ml of cold 10% trichloroacetic acid was added to 1 ml of the homogenate. The precipitate was washed three times with and suspended in 5% trichloroacetic acid, and heated at 90°C for 15 min to solubilize the DNA. After centrifugation at 3000 rpm for 10 min, 1 ml of the supernatant was mixed with 9 ml toluene-based scintillator containing 0.01% PPO, 0.4% POPOP and 33% Triton X-100. The radioactivity was measured using a Packard Tri-Carb 460. The amount of DNA was assayed according to Burton [7].

2.4. *Estimation of intracellular polyamine level*

Liver was homogenized with 4 vols of 5% trichloroacetic acid. After centrifugation at 30 000 × g for 30 min, 5 μl portions of the supernatant were analyzed for polyamines with a Shimadzu LC-3A liquid chromatogram using a cation-exchange column (ISC-05). The column was eluted with 0.2 N sodium citrate containing 2.3 M NaCl and 2% n-propanol. Polyamines separated by the column were reacted with o-phthalaldehyde and were measured using fluorescence.

3. RESULTS

Fig.1 shows the course of [^{3}H]thymidine incorporation into DNA after partial hepatectomy. Incorporation increased and peaked at 36 h. IFN administration suppressed the incorporation at 36 h to 30% of that of the control mice and delayed the peak until 48 h. To find why IFN suppresses DNA synthesis in the regenerating liver, we tested the effect of IFN on the intracellular level of polyamines, which are important in cell growth. Fig.2 shows that the putrescine level increased 8 h after the partial hepatectomy, peaked at 24 h, and then decreased. IFN suppressed the elevation of the putrescine level caused by partial hepatectomy. The increases in spermidine at 24 h and 48 h after the operation were significant (both $p<0.01$) compared to the level of sham-operated mice. Unlike putrescine, the spermidine level was not affected by IFN. The spermine level had not changed 48 h after the partial hepatectomy and was not influenced by IFN (not shown). These results suggest a close correlation between the inhibition of putrescine synthesis and that of DNA synthesis.

To ascertain that the inhibition by IFN of DNA synthesis is mediated by the inhibition of putrescine synthesis, we examined the effect of exogenous putrescine on the inhibition of DNA syn-

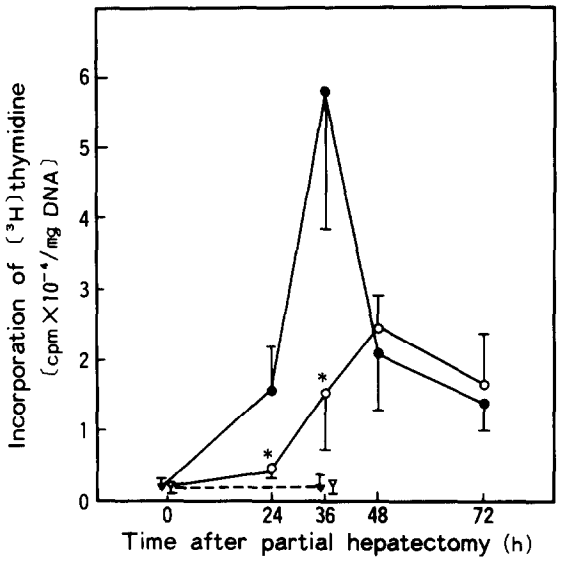

Fig.1. [^{3}H]Thymidine incorporation into DNA after partial hepatectomy. IFN (3×10^4 IU) was given i.p. 16 h before partial hepatectomy. [^{3}H]Thymidine (10 μCi/100 g body wt) was injected i.p. 4 h before the times indicated. Data shown are means ± SD for 4–6 animals. (●) Partially hepatectomized mice not given IFN, (○) partially hepatectomized mice given IFN, (▼) sham-operated mice not given IFN and (▽) sham-operated mice given IFN. (*) $p<0.05$, significant difference was compared to the partially hepatectomized mice not given IFN.

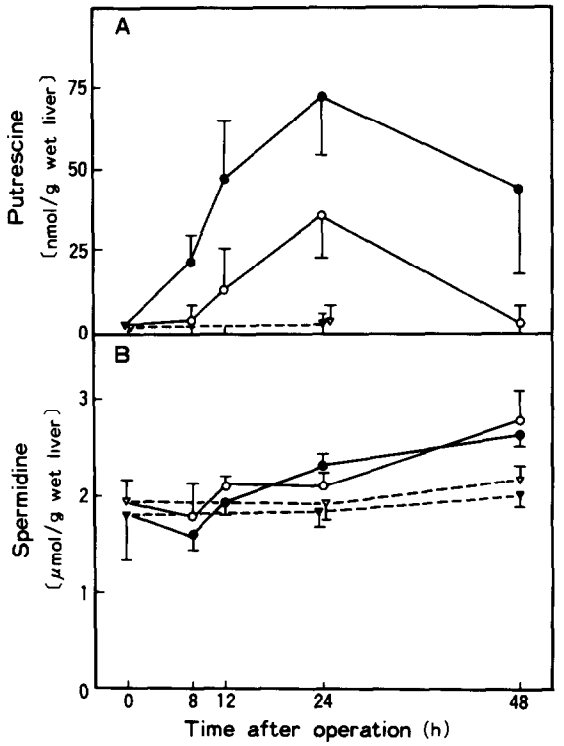

Fig.2. Effects of IFN on intracellular levels of putrescine (A) and spermidine (B): IFN (3×10^4 IU) was given i.p. 16 h before partial hepatectomy. Polyamines were extracted from the remaining liver at the times indicated. Data shown are mean ± SD for 4–6 animals. For symbols see legend to fig.1.

thesis. Fig.3 shows that the increase in [^{3}H]thymidine incorporation into DNA 36 h after partial hepatectomy was suppressed by IFN and that the intraperitoneal injection of putrescine blocked the suppression. Putrescine did not affect [^{3}H]thymidine incorporation into the DNA of the liver of sham-operated mice.

IFNs increase the intracellular level of 2′,5′-oligoadenylate (2-5A), which is probably involved in the antiviral action of IFN. To see whether the inhibition by IFN of DNA synthesis in regenerating liver arises from 2-5A, we used core 2-5A to find out whether it also reduces the intracellular level of putrescine and inhibits DNA synthesis in regenerating liver. Core 2-5A at the dose of 0.1 mg given immediately and again 2 h after partial hepatectomy suppressed the elevation of the putrescine level, but not that of spermidine or spermine (table 1). A higher dose of core 2-5A,

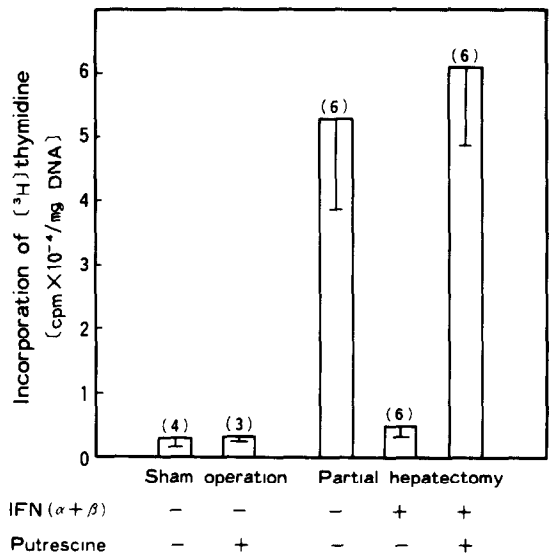

Fig.3. Effect of putrescine on inhibition by IFN on DNA synthesis. IFN (3×10^4 IU) was given to mice i.p. 16 h before partial hepatectomy. Putrescine (1 mg) was injected i.p. three times, 8, 16, and 24 h after the operation. Mice were labelled with [^{3}H]thymidine (10 μCi/100 g body wt) injected i.p. 32 h after the operation and were killed 4 h later. Data shown are mean ± SD. The numbers in parentheses give the number of animals.

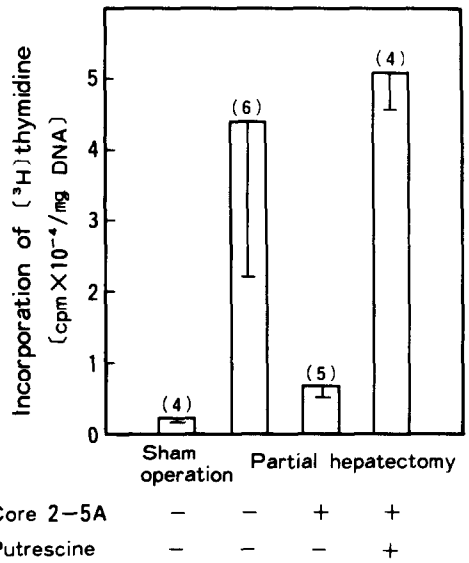

Fig.4. Effects of core 2-5A and putrescine on [^{3}H]thymidine incorporation into DNA. Core 2-5A (0.1 mg) instead of IFN was given to mice i.p. immediately and again 2 h after partial hepatectomy. Other experimental conditions were as in fig.3. Data shown are mean ± SD. The numbers in parentheses give the number of animals.

Table 1

Effect of core 2-5A on intracellular levels of polyamines

Treatment	Polyamines (nmol/g wet liver)		
	Putrescine	Spermidine	Spermine
Sham operation	11.0 ± 5.4 (5)	1786.2 ± 292.6 (5)	1387.4 ± 220.9 (5)
Partial hepatectomy	109.4 ± 36.5 (7)	1661.6 ± 373.3 (7)	996.2 ± 258.5 (7)
Partial hepatectomy + core 2-5A (0.1 mg)	45.8 ± 16.6 (6)	1753.1 ± 558.3 (6)	1087.9 ± 380.1 (6)
Partial hepatectomy + core 2-5A (0.3 mg)	25.2 ± 18.7 (5)	1288.2 ± 220.0 (5)	788.8 ± 187.4 (5)

Core 2-5A was given to mice i.p. immediately and again 2 h after partial hepatectomy. The mice were killed 24 h after the operation and polyamines were extracted from the remaining liver and assayed as described in the text. Each value is the mean ± SD for 5-7 animals. The numbers in parentheses give the number of animals

0.3 mg, decreased not only putrescine but also spermidine and spermine levels 24 h after the operation. Stimulation of DNA synthesis was inhibited by core 2-5A and this inhibition was prevented by administration of putrescine (fig.4).

4. DISCUSSION

Studies of IFN inhibition of cell growth have shown a close relationship between the inhibition by IFN of polyamine synthesis and that of cell growth. IFN inhibits the induction of two enzymes for polyamine biosynthesis, ornithine decarboxylase [4,5] and *S*-adenosylmethionine decarboxylase [8]. The combination of IFN and α-difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase, synergistically inhibits cell growth [9,10]. However, the inhibition of cell growth is not always associated with statistically significant alterations in intracellular levels of polyamines [10,11]. Moreover, addition of putrescine to culture medium does not reverse the antiproliferative effect of IFN [8,11], suggesting that inhibition of enzymes key to polyamine synthesis participates in the mechanism of the antiproliferative effect of IFN, but is not the whole mechanism. However, our results presented here showed that IFN administration suppressed accumulation of putrescine and also the stimulation of DNA synthesis in the liver induced by partial hepatectomy, and that the suppression of DNA synthesis was completely reversed by putrescine. These results indicate that IFN inhibits putrescine synthesis, resulting in the suppression of DNA synthesis.

The intracellular mediator of the antiproliferative action of IFN has been unknown. IFNs induce several proteins including double-stranded RNA-dependent protein kinase and 2-5A synthetase, which may be involved in the antiviral action of IFN [1]. It is probable that these two enzymes are most important to the antiproliferative action of IFN. The following results support the suggestion that 2-5A participates in the antiproliferative action of IFN. Core 2-5A inhibits DNA synthesis of mouse spleen lymphocytes stimulated by concanavalin A [12]. 2-5A synthetase activity of rat liver decreases rapidly after partial hepatectomy [13]. However, no correlation between 2-5A synthetase activity and cell growth was observed in human cells [11,14,15]. Here we showed that the effects of core 2-5A are very similar to those of IFN. Core 2-5A as well as IFN inhibits both the accumulation of putrescine and

the stimulation of DNA synthesis. The suppression of DNA synthesis is reversed by putrescine. These results suggest that 2-5A is important for the antiproliferative action of IFN.

In conclusion, IFN increases the intracellular level of 2-5A by inducing 2-5A synthetase activity, and the oligoadenylate inhibits accumulation of putrescine through suppression of ornithine decarboxylase induction, resulting in the inhibition of DNA synthesis in the regenerating liver.

ACKNOWLEDGEMENTS

We wish to thank Miss Yasuko Mimura for her excellent technical assistance and Miss Caroline Latta for critical reading of the manuscript. We are also grateful to Dr S. Kobayashi of the Basic Research Laboratories of Toray Industries, Kamakura for giving us mouse interferon.

REFERENCES

[1] Lengyel, P. (1982) Annu. Rev. Biochem. 51, 251-282.
[2] Clemens, M.J. and McNurlan, M.A. (1985) Biochem. J. 226, 345-360.
[3] Tabor, C.W. and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749-790.
[4] Sreevalsan, T., Taylor-Papadimitriou, J. and Rozengurt, E. (1979) Biochem. Biophys. Res. Commun. 87, 679-685.
[5] Sekar, V., Atmar, V.J., Joshi, A.R., Kim, M. and Kuehn, G.D. (1983) Biochem. Biophys. Res. Commun. 114, 950-954.
[6] Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 13, 186-202.
[7] Burton, K. (1956) Biochem. J. 62, 315-323.
[8] Lee, E. and Sreevalsan, T. (1981) Adv. Polyamine Res. 3, 175-186.
[9] Sunkara, P.S., Parkash, N.J., Mayer, G.D. and Sjoerdma, A. (1983) Science 219, 851-853.
[10] Heston, W.P.W., Fleischmann, J., Tackett, R. and Ratliff, T.L. (1984) Cancer Res. 44, 3220-3225.
[11] Chapekar, M.S. and Glazer, R.I. (1984) Cancer Res. 44, 2144-2149.
[12] Kimchi, A., Shure, H. and Revel, M. (1979) Nature 282, 849-851.
[13] Etienne-Smekens, M., Vanderbussche, P., Content, J. and Dumont, J.E. (1983) Proc. Natl. Acad. Sci. USA 80, 4609-4613.
[14] Tomita, Y., Cantell, K. and Kuwata, T. (1982) Int. J. Cancer 30, 161-165.
[15] Verhaegen-Lewalle, M., Kuwata, T., Zhang, Z.-X., DeClerq, E., Cantell, K. and Content, J. (1982) Virology 117, 425-434.