ORIGINAL ARTICLE

Antitumor Effects of an Interferon-Loaded Silicone Formulation in Human Renal Cell Carcinoma in Nude Mice

Ken Marumo,¹ Yongping Huang^{1,2} and Masaru Murai¹

¹ Department of Urology, School of Medicine, Keio University,

² Human Gene Sciences Center, Tokyo Medical and Dental University, Tokyo, Japan

(Received for publication on June 15, 1999)

Abstract. Conventional therapy for renal cell carcinoma using interferon (IFN) has shown limited antitumor action. The IFN-loaded silicone formulation (IFN-SF) is a sustained-release formulation of human lymphoblastoid IFN, with a medical grade silicone elastomer used as the carrier material. We evaluated the antitumor effect of IFN-SF on human renal cell carcinoma cell line (KU-2) transplanted to nude mice. The treatment was started when the tumor nodules had grown 6 to 8 mm in diameter. IFN-SF, or an aqueous solution of IFN, was given by subcutaneous or peritumoral injection. Antitumor effects were evaluated according to tumor weights calculated as $(\text{long diameter}) \times (\text{short diameter})^2/2$ in 7 groups consisting of 6 mice each. Serum IFN levels remained detectable up to 30 days after subcutaneous injection of IFN-SF. IFN-SF administered by the subcutaneous and peritumor route significantly inhibited growth of the tumor when compared with tumor growth in the untreated mice and mice treated with aqueous IFN solution. IFN-SF had equivalent inhibitory effects on tumor growth by peritumor and subcutaneous injection. Results indicated that the use of IFN-SF could reduce the frequency of injections and provide better treatment of patients with renal cell carcinoma because of its long-acting effect when it is used systemically. (Keio J Med 48 (4): 179–183, December 1999)

Key words: interferon, drug delivery system, silicone, renal cell carcinoma, nude mouse

Introduction

Renal cell carcinoma is a relatively common tumor, that is expected to afflict from 2,500 to 3,700 people in Japan every year,¹ and 2,993 deaths from metastatic disease were registered in 1997 in Japan.² Radical nephrectomy remains the treatment of choice for patients who present with clinically localized disease, but approximately 5% of patients will have local disease recurrence after surgery and 25% develop distant metastases.³ Once the disease has become metastatic or recurrent, renal cell carcinoma carries a poor prognosis with 74% mortality at 1 year and 96% mortality at 3 years.⁴ This is due to the absence of effective therapy for patients with advanced renal cell carcinoma. Although interferon (IFN) is known to produce tumor regression in patients with metastatic renal cell carcinoma, conventional systemic IFN therapy has only a limited antitumor efficacy, the response rate being between 5% and 26%.^{5–9} In addition, there are some concerns about the clinical use of IFN; the administered

IFN is cleared rapidly with an initial half-life of less than 3 hours and causes a number of adverse reactions such as fever, fatigue, appetite loss, bone marrow suppression and hepatotoxicity.¹⁰

These adverse reactions would be attenuated, and the antitumor effects would be improved, if a long-acting IFN preparation could be clinically used with systemic administration, or such a preparation could be applied to the local target tissue or organs. We evaluated the antitumor effect of a drug delivery system on human renal cell carcinoma transplanted in nude mice, utilizing the newly developed sustained-release IFN-loaded silicone formulation (IFN-SF).

Materials and Methods

Nude mice

Male athymic nude mice (nu/nu, BALB/c background), purchased from Shizuoka Experimental Animal Center (Hamamatsu, Japan), were housed in a

negative-pressure safety rack (CLEA Japan Inc, Tokyo, Japan) under specific pathogen-free conditions. The animals were given germ-free mouse food, CL-2 (CLEA Japan Inc, Tokyo, Japan) and sterilized distilled water, to prevent infection. In all experiments, 6-week-old mice were used.

Tumor cells

The human renal cell carcinoma tumor cell line, KU-2, was derived from a pulmonary metastatic lesion of a renal cell carcinoma capable of producing erythropoietin. After having been transplanted to nude mice, KU-2 was established as a cell line in 1975.^{11,12} The KU-2 tumor masses were excised and cut into 3 mm × 3 mm pieces; 1 piece was implanted subcutaneously into the back of each nude mouse with a trocar needle.

IFN solution

Human lymphoblastoid IFN, a human natural alpha IFN, was a product of Sumitomo Pharmaceuticals Co, Ltd. (Osaka, Japan). Human lymphoblastoid IFN was derived from Namalwa cells, and its specific activity was more than 1×10^8 IU/mg protein. The aqueous solution of IFN (3×10^6 IU/ml) was administered at a dose of 1×10^5 IU per injection.

Silicone formulation

The IFN-loaded silicone formulation (IFN-SF) was developed by Sumitomo Pharmaceuticals Co, Ltd. Medical grade silicone elastomer (Dow Corning Corporation, Midland, Mich, USA) was used as the carrier material. IFN-SF was produced by extrusion molding without use of any organic solvent or heat. The IFN-SF is a rod-shaped pellet, 1.8 mm in diameter and 10 mm in length, containing 1×10^6 IU, 1×10^7 IU or 1×10^8 IU of human lymphoblastoid IFN (Fig. 1). The embedded IFN is released through interconnecting channels and cracks, which gradually form after the silicone formulation is placed under water (Fig. 2). The rate of drug release can be controlled by the composition of the formulation. The IFN-SF can be administered by standard injection with a trocar needle.

Experimental therapy model

When the tumor nodules had grown 6 to 8 mm in diameter, 20 days after implantation, the nude mice were randomized into 7 groups consisting of 6 mice per group, and the treatment was started. The groups were as follows: (1) no treatment (control); (2) peritumoral injection of placebo-SF; (3) subcutaneous injection of IFN solution (1×10^6 IU); (4) subcutaneous injection of IFN-SF (1×10^6 IU and 1×10^8 IU); (5) peritumoral

Fig. 1 The interferon-loaded silicone formulation is 1.8 mm in diameter and 10 mm in length. Medical grade silicone elastomer was used as the carrier material. Each silicone formulation contains 1×10^6 IU, 1×10^7 IU or 1×10^8 IU human lymphoblastoid interferon.

Fig. 2 The interferon-loaded silicone formulation in the covered-rod form is composed of a core of silicone, which contains interferon, and an outer silicone layer. Interferon is released from both ends of the rod through interconnecting channels in the silicone core.

injection of IFN-SF (1×10^6 IU and 1×10^8 IU).

The aqueous solution was administered subcutaneously 10 times daily at a dose of 1×10^5 IU at sites distant from the tumor from 20 days after transplantation of the tumor. IFN-SF was administered subcutaneously at sites distant from the tumor, or by peritumoral injection, at 20 days after transplantation of the tumor. The peritumoral injections were given at sites close to the tumor, not directly into the tumor, to minimize mechanical injury to the tumor. The mice treated by subcutaneous injections served as a model of systemic administration of IFN.

Measurement of tumor growth

The perpendicular diameters of the tumor were measured daily until 60 days after tumor transplantation with a sliding caliper, and the tumor weights were estimated by the following formula: (long diameter) × (short diameter)²/2.

Determination of IFN concentrations

The serum IFN concentrations were determined after the subcutaneous injection of IFN solution containing 1×10^5 IU, and IFN-SF containing 1×10^6 IU, 1×10^7 IU, or 1×10^8 IU of IFN. IFN concentrations in the tumor tissue were determined by radioimmunoassay (RIA) (IFN- α kit, Dainabot Radioisotope Laboratories Ltd, Tokyo, Japan) after the subcutaneous injection of IFN solution containing 1×10^5 IU or IFN-SF containing 1×10^6 IU, at various time points after administration. Human lymphoblastoid IFN (Sumitomo Pharmaceuticals Co Ltd, Lot No. 631138) was used as a standard.

Blood was drawn from the retro-orbital sinus of the mouse, and the tumor tissue was extirpated surgically under light ether anesthesia. The tumor nodule was homogenized in phosphate-buffered saline. IFN concentration in supernatant was determined by RIA for the determination of IFN. The concentration of IFN was expressed as the average value for 3 mice. The mice used in the determination of IFN concentrations were not in any of the 6 experimental therapy groups.

Statistical analysis

Representative data from each experiment is presented as the mean values $\pm$ standard deviation or standard error, as described in the tables. For statistical analysis, repeated ANOVA measures were conducted, and, provided a significant group difference existed, Fisher's protected least significant difference *post-hoc* procedure was applied to compare different groups of treated mice. A value of $p < 0.05$ was considered to be significantly different. All analyses were completed using Stat View 5.0 for Macintosh (SAS Institute Inc., Cary, NC, USA).

Results

Pharmacokinetics of IFN

The IFN serum concentrations after subcutaneous administration of IFN solution were maintained at over 2,000 IU/ml for 3 hours after treatment ($2,590 \pm 140$ IU/ml in 1 hour, $2,440 \pm 170$ IU/ml in 2 hours, $2,680 \pm 140$ IU/ml in 3 hours), subsequently declined, and were still detectable up to 24 hours after administration (150 ± 27 IU/ml). The tumor tissue IFN level after subcutaneous injection of the IFN solution peaked 1 hour after treatment and returned to the pretreatment level within 24 hours (Fig. 3). The IFN serum concentration after subcutaneous administration of IFN-SF peaked 1 day after treatment (127 ± 22 IU/ml by 1×10^6 IU of IFN-SF, 496 ± 58 IU/ml by 1×10^7 IU of IFN-SF, $39,800 \pm 688$ IU/ml by 1×10^8 IU of IFN-SF),

subsequently declined, and was still detectable up to 30 days after administration (10.5 ± 3.1 IU/ml by 1×10^6 IU of IFN-SF, 28.0 ± 8.7 IU/ml by 1×10^7 IU of IFN-SF, 239 ± 18.0 IU/ml by 1×10^8 IU of IFN-SF) (Fig. 4).

The tumor tissue IFN levels after administration of IFN-SF differed between the two routes of administration. The tumor tissue IFN level peaked 2 days after injection of IFN-SF by both routes of administration (269 ± 107 IU/mg by subcutaneous injection, $1,370 \pm 680$ IU/mg by peritumoral injection), and the concentration of IFN was 5 times higher in mice treated by peritumoral injection than that in mice treated by subcutaneous injection of IFN-SF. However, the high IFN concentration, produced by peritumoral injection of IFN-SF decreased rapidly within 4 to 10 days after the treatment (Fig. 5).

Fig. 3 Interferon levels in serum and tumor tissue after subcutaneous administration of the aqueous solution of 10^5 IU human lymphoblastoid interferon. The concentration of IFN is expressed as the average value and standard error for three mice in IU/ml serum and IU/mg tumor, ^b not detectable (pretreatment level).

Fig. 4 Serum interferon levels after subcutaneous administration of the interferon silicone formulation. The IFN concentration is expressed as the average value and standard error for three mice in IU/ml serum.

Antitumor activity

Effects of an aqueous solution of IFN or IFN-SF on tumor growth are shown in Fig. 6. The aqueous solution of IFN administered subcutaneously 10 times daily by injection at a dose of 1×10^5 IU significantly inhibited growth of the tumor ($p < 0.01$). Administration of IFN-SF demonstrated significantly more potent tumor growth inhibition of the tumor by both subcutaneous and peritumoral injection compared to tumor growth in mice treated by IFN solution ($p < 0.01$). There was no statistically significant difference in the potency of tumor growth inhibition between the peritumoral and subcutaneous injection of IFN-SF (1×10^6 IU, $p = 0.44$; 1×10^8 IU, $p = 0.25$, respectively).

Fig. 5 Tumor tissue interferon levels after subcutaneous or peritumoral administration of the interferon silicone formulation containing 10^6 IU of human α -interferon. The IFN concentration is expressed as the average value and standard error for three mice in IU/mg tumor.

Fig. 6 Effects of an aqueous solution of interferon and the interferon-loaded silicone formulation on the growth of KU-2 tumors in nude mice. Aqueous IFN solution (IFN-Sol) was administered subcutaneously 10 times daily at a dose of 10^5 IU/mouse; interferon-loaded silicone formulation (IFN-SF) containing 10^6 IU or 10^8 IU of human α -interferon was administered by subcutaneous (SC) or peritumoral (PT) injection; the weight of tumors is expressed as the average value and standard deviation for 6 mice; ^a $p < 0.01$ vs untreated mice; ^b $p < 0.01$ vs IFN-Sol.

The dose-dependent antitumor effects of subcutaneous and peritumoral injection of IFN-SF were studied using silicone formulations containing different doses of IFN. A 1×10^8 IU dose of IFN-SF was not shown to have any significantly greater inhibitory effect on tumor growth than a 1×10^6 IU dose (subcutaneous injection, $p = 0.16$; peritumoral injection, $p = 0.08$).

Discussion

To evaluate the clinical potential of IFN-SF in the treatment of advanced renal cell carcinoma, the pharmacokinetics of IFN and its growth inhibitory effect on tumors implanted in nude mice were studied. The antitumor activity of the IFN-SF was studied using human renal cell carcinoma KU-2 cells, which are sensitive to IFN therapy.^{13,14}

Although the relationship between the antitumor action of IFN- α and its pharmacokinetics has not been completely clarified, a sustained-release formulation of the drug could potentially reduce the frequency of injections and may improve local or intralesional treatment of patients with malignant diseases. Eppstein¹⁵ reported increased localized retention and altered distribution of IFN in a mouse model after intramuscular or intravenous injection of human IFN- α encapsulated in liposomes. Rosenblum *et al.*¹⁶ showed that an immune complex, prepared by mixing human leukocyte IFN with an anti-IFN monoclonal antibody, prevented plasma clearance and degradation of IFN after intravenous injection in rats. Edwards *et al.*¹⁷ demonstrated promising clinical results in treatment of basal cell carcinoma by intralesional injection of a sustained-release protamine zinc chelate IFN formulation.

We determined the time course of serum IFN concentrations in mice after subcutaneous injection of silicone formulations containing 1×10^6 IU, 1×10^7 IU or 1×10^8 IU of human lymphoblastoid IFN. IFN in mouse serum reached a peak level 1 day after administration, and was detectable for more than 30 days. After subcutaneous administration of an aqueous solution of human lymphoblastoid IFN, the IFN levels in serum peaked within 1 hour, and declined rapidly thereafter to become undetectable in tumor tissue by 24 hours after administration. The present study confirmed the antitumor effect of human lymphoblastoid IFN, with daily subcutaneous administration of human lymphoblastoid IFN solution at a dose of 1×10^5 IU significantly inhibiting the growth of KU-2 tumors in nude mice. The effects of peritumoral injection of IFN solution were not evaluated, because such mode of treatment is not clinically practical. We previously reported that the IFN minipellet, a sustained release formulation of human lymphoblastoid IFN comprising atelocollagen used as the carrier material, showed greater inhibition of tumor growth by peritumoral injection than subcutaneous in-

jection.¹⁸ From these results, it was assumed that administration of IFN-SF to the tumor site, which has a greater long acting effect than the IFN minipellet, would give more favorable results in the treatment of this tumor.

The antitumor effect of IFN-SF was found to be significantly more potent than the IFN solution, but there was no significant difference in suppression of tumor growth between the peritumoral and subcutaneous injection of IFN-SF. A possible explanation is that IFN was released from both ends of the silicone formulation rod, and the IFN thus did not perfuse efficiently into the tumor tissue even when it was injected in an area very close to the tumor. The results may also be explained by the fact that IFN levels in the tumor tissue after peritumoral injection were maintained at higher levels in the initial 5 days, while the levels decreased to the same as those in the subcutaneous injection group 10 days after administration.

In the present study, no dose-dependent antitumor effects were observed in either treatment by subcutaneous injection or by peritumoral injection. Reports from clinical trials have also suggested that renal cell carcinoma does not always respond to IFN therapy in a dose-dependent manner. Trump *et al.*¹⁹ treated 35 patients with high-dose lymphoblastoid IFN with a daily maximal dose of 30×10^6 IU/mm², and got a response rate of 13%. Marshall *et al.*²⁰ reported that 4 of 16 patients (25%) responded to daily administration of recombinant IFN- α at a dose of 30×10^6 IU.

We studied the antitumor effect of IFN-SF using human IFN and human renal cancer cells in athymic nude mice. Since the action of IFN is species-specific, the antitumor effects observed in these studies were mainly due to the direct antiproliferative effects on the tumor cells. When the IFN minipellet is administered to a human subject, additional antitumor effects due to stimulation of a specific immune reaction between the tumor cells and the host immune system at the tumor site can be expected.

Using a new formulation of human lymphoblastoid IFN, IFN-SF, we successfully treated renal cell carcinoma transplanted to nude mice. The pharmacokinetics showed that IFN was detectable in the serum even 30 days after administration of IFN-SF by the subcutaneous route. The results indicate that the IFN-SF will be effective as a sustained-release formulation of IFN in the treatment of patients with renal cell carcinoma.

References

- Kotake T, Kinouchi T: Characterization of renal cell carcinoma: current topics. *Gan To Kagaku Ryoho* (Jpn J Cancer Chemother) 1994; 21: 5–11 (in Japanese)
- Statistics and Information Department, Minister's Secretariat, Ministry of Health and Welfare: Cause of death (General mortality). In: *Vital statistics of Japan 1997*, volume 3, Tokyo, Health and Welfare Statistics Association, 1999; 116–533 (in Japanese)
- Rabinovitch RA, Zelefsky MJ, Gaynor JJ, Fuks Z: Patterns of failure following surgical resection of renal cell carcinoma: implications for adjuvant local and systemic therapy. *J Clin Oncol* 1994; 12: 206–212
- Patel NP, Lavengood RW: Renal cell carcinoma: natural history and results of treatment. *J Urol* 1978; 119: 722–726
- Quesada JR, Swanson DA, Trindade A, Gutterman JU: Renal cell carcinoma: antitumor effects of leukocyte interferon. *Cancer Res* 1983; 43: 940–947
- deKernion JB, Sarna G, Figlin R, Lindner A, Smith RB: The treatment of renal cell carcinoma with human leukocyte alpha-interferon. *J Urol* 1983; 130: 1063–1066
- Marumo K, Murai M, Hayakawa M, Tazaki H: Human lymphoblastoid interferon therapy for advanced renal cell carcinoma. *Urology* 1984; 24: 567–571
- Kirkwood JM, Harris JE, Vera R, Sandler S, Fischer DS, Khandekar J, Ernstoff MS, Gordon L, Lutes R, Bonomi P, *et al.*: A randomized study of low and high doses of leukocyte α -interferon in metastatic renal cell carcinoma: the American Cancer Society collaborative trial. *Cancer Res* 1985; 45: 863–871
- Quesada JR, Kurzrock R, Sherwin SA, Gutterman JU: Phase II studies of recombinant human interferon gamma in metastatic renal cell carcinoma. *J Biol Response Mod* 1987; 6: 20–27
- Marumo K, Hayakawa M, Murai M, Tazaki H: Immunological study of anti-cancer effects of human alpha interferon for advanced renal cell carcinoma. *Nippon Hinyokika Gakkai Zasshi* (Jpn J Urol) 1985; 76: 965–973 (in Japanese)
- Katsuoka Y, Baba S, Hata M, Tazaki H: Transplantation of human renal cell carcinoma to the nude mice: as an intermediate of in vivo and in vitro studies. *J Urol* 1976; 115: 373–376
- Katsuoka Y: Functional and morphological observation of human renal cell carcinoma utilizing nude mouse and cell culture system. *Nippon Hinyokika Gakkai Zasshi* (Jpn J Urol) 1978; 69: 285–303 (in Japanese)
- Marumo K: Cytotoxic effects of human alpha interferon against human renal cancer cell line (KU-2). *Keio J Med* 1982; 31: 7–20
- Yamaoka T, Takada H, Yanagi Y, Kataoka T, Sakurai Y: The antitumor effects of human lymphoblastoid interferon on human renal cell carcinoma in athymic nude mice. *Cancer Chemother Pharmacol* 1985; 14: 184–187
- Eppstein DA: Altered pharmacologic properties of liposome-associated human interferon-alpha. II. *J Interferon Res* 1982; 2: 117–125
- Rosenblum MG, Unger BW, Gutterman JU, Hersch EM, David GS, Frincke JM: Modification of human leukocyte interferon pharmacology with a monoclonal antibody. *Cancer Res* 1985; 45: 2421–2424
- Edwards L, Tucker SB, Perednia D, Smiles KA, Taylor EL, Tanner DJ, Peets E: The effects of an intralesional sustained-release formulation of interferon alpha-2b on basal cell carcinomas. *Arch Dermatol* 1990; 126: 1029–1032
- Marumo K, Oya M, Murai M: Application of the interferon minipellet to human renal cell carcinoma in nude mice. *Int J Urol* 1997; 4: 55–61
- Trump DL, Elson PJ, Borden EC, Harris JE, Tuttle RL, Whisnant JK, Oken MM, Carignan JR, Ruckdeschel JC, Davis TE: High-dose lymphoblastoid interferon in advanced renal cell carcinoma: an Eastern Cooperative Oncology Group Study. *Cancer Treat Rep* 1987; 71: 165–169
- Marshall ME, Simpson W, Butler K, Fried A, Fer M: Treatment of renal cell carcinoma with daily low-dose alpha-interferon. *J Biol Response Mod* 1989; 8: 453–461