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The antitumor effects of human lymphoblastoid interferon on human renal cell carcinoma in athymic nude mice

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Summary. *The antitumor effects of human lymphoblastoid interferon (HLBI) on human renal cell carcinomas transplanted in nude mice, i.e., KU-2 and RCC-1, were investigated and compared with those on other human tumors, viz. HeLa (cervical carcinoma), KB (nasopharyngeal carcinoma), H.Ep#2 (laryngeal carcinoma), and MX-1 (breast cancer). A pharmacokinetic study on HLBI was also carried out in non-tumor-bearing nude mice.*

HLBI therapy was performed with a dose of 10^5 IU/mouse by daily SC or IT (intratumoral) injection for 2–4 weeks. Two renal cell carcinomas, KU-2 and RCC-1, proved to be highly sensitive to HLBI. The growth of these tumors was inhibited not only by IT but also by SC injection of HLBI. In contrast, HLBI exerted only a slight effect or none at all on the other human tumors, namely, MX-1, KB, H.Ep#2, and HeLa, even when given by IT injection. The data show that the antitumor effects of HLBI depend on the types of human tumors and may be relevant to the clinical observation that renal tumors are sensitive to HLBI.

The serum HLBI reached a peak level of 4,390 U/ml 1 h after a single SC injection at a dose of 10^5 IU/mouse and declined with a half-life of 4 h to 128 U/ml 24 h later. This time-course was not affected by 10 consecutive daily injections of HLBI. In nude mice, the consecutive administration of HLBI at this dose level appears to result in neither accumulation nor rapid clearance due to antibody formation. From this range of serum HLBI levels and its in vitro anticellular activity, the in vivo antitumor effects of HLBI in nude mice seemed to depend on its direct anticellular action.

Introduction

Extensive clinical testing of human leukocyte and lymphoblastoid interferons (α-type interferons) has been carried out to date, and the activities of these agents against several tumors have been elucidated [1, 2, 5, 6]. Recently it was noted that patients with refractory metastatic renal cell carcinoma were effectively treated with α-type interferons [4, 7]. These facts led us to plan to evaluate the antitumor effects of human lymphoblastoid interferon (HLBI) on human renal cell carcinomas in an experimental system.

The nude mouse-human tumor xenograft system was chosen as a suitable experimental model for this purpose, for the following two reasons. First, the actions of interferons are species-specific. Secondly, this in vivo experimental system is considered to be more predictable as regards the clinical effects of interferons than the in vitro culture system using human tumor cells because the human tumors grown in nude mice conserve the nature of the original tumors to a large extent.

In this work, the growth-inhibitory effects of HLBI on two renal cell carcinomas, KU-2 and RCC-1, transplanted in nude mice were examined and compared with those on four other types of human tumors, MX-1 (breast cancer), H.Ep#2 (laryngeal carcinoma), HeLa (cervical carcinoma), and KB (nasopharyngeal carcinoma). In addition, the pharmacokinetics of HLBI in nude mice was investigated.

Materials and methods

1. Nude mice. Male athymic nude mice (nu/nu, BALB/c background) were purchased from CLEA Japan Inc., Tokyo, and were housed in a negative-pressure safety rack (CLEA Japan Inc., Tokyo) under specific pathogen-free conditions. The animals were given germ-free mouse food (CL-2, CLEA Japan Inc., Tokyo) and sterilized distilled water to prevent infections. For all experiments, 5- to 6-week-old mice were used.

2. Human tumors. MX-1 and H.Ep#2 were supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo. RCC-1 was provided by the Central Institute for Experimental Animals, Kanagawa, Japan [8]. KU-2 was a kind gift from Prof. H. Tazaki, Department of Urology, School of Medicine, Keio University [3]. These tumors were maintained by serial transplantation in nude mice. KB and HeLa were purchased from the Divison of Laboratory Products, Dainippon Pharmaceutical Co. Ltd, Osaka, Japan, and were serially passaged in cell cultures.

3. Human lymphoblastoid interferon (HLBI). Two lots of HLBI, LE-007 and CIN/37, were used. Lot No. LE-007 was a product of Sumitomo Chemical Co. Ltd, Osaka, Japan, and was purified from crude interferon supplied by Wellcome Research Laboratories, Beckenham, Kent, Great Britain. Lot No. CIN/37 was a product of Wellcome Research Laboratories. These two preparatations had a specific activity of more than 10^8 IU/mg protein and were of the same composition. Interferon titers were 5.13×10^6 IU/ml for LE-007 and 6.13×10^6 IU/ml for CIN/37. LE-007 and CIN/37 were diluted to 10^6 IU/ml with phosphate-buffered saline (−) containing 10% normal mouse serum, and the aliquots were stored at −20° C

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until use. The vehicles for HLBI preparations were diluted and stored in the same way.

4. Tumor transplantation. The masses of KU-2, RCC-1, MX-1, and H.Ep#2 tumors were excised and cut into about 3 mm × 3 mm pieces. Then each fragment was implanted SC at a ventral site in a nude mouse with a trocar needle. KB and HeLa, which had been serially passaged in cell cultures, were suspended in minimum essential medium containing 10% fetal calf serum, and 2×10^6 cells were injected to a nude mouse at a ventral site with a 25-gauge needle.

5. HLBI therapy. When tumor nodules grew to 5–8 mm in diameter, nude mice were separated into groups consisting of six to nine mice after randomization, and HLBI therapy was started. HLBI (10^6 IU/ml) was injected IT or SC to nude mice at a dose of 10^5 IU/mouse unless otherwise stated. The IT injections were given directly into the tumors that had been implanted SC to the nude mice at a ventral site, while the SC injections were given at sites distant from the tumors. A control group was given the vehicle in the same way. HLBI therapy was performed daily for 2–4 weeks.

6. Measurement of tumor growth. The length and width of the tumors were measured twice a week with a sliding caliper, and the estimated tumor volume (V) was expressed as V = length (mm) × [width (mm)]^2. On the 7 day after the final administration of HLBI the animals were sacrificed, and the tumors were excised and weighed. The statistical analysis was carried out by Mann-Whitney's U-test.

7. Measurement of serum HLBI levels. The serum HLBI levels were measured by radioimmunoassay, using an Interferon-α kit (Dainabot Radioisotope Lab. Ltd, Japan). Human lymphoblastoid interferon (Lot No. KN-001, a product of Sumitomo Chemical Co. Ltd, Japan) was used as a standard.

Nude mice were divided into two groups, that is, a single-injection group and a consecutive-injection group, and the serum specimens were withdrawn as follows to measure HLBI concentrations. In the single-injection group, HLBI was injected SC to nude mice at a dose of 10^5 IU/mouse, and the serum was obtained 0.5, 1, 3, 7, and 24 h later. In the consecutive-injection group, the SC injections were given daily at a dose of 10^5 IU/mouse for 10 days, and the serum was obtained 0.5, 1, 3, 7, and 24 h after the final injection.

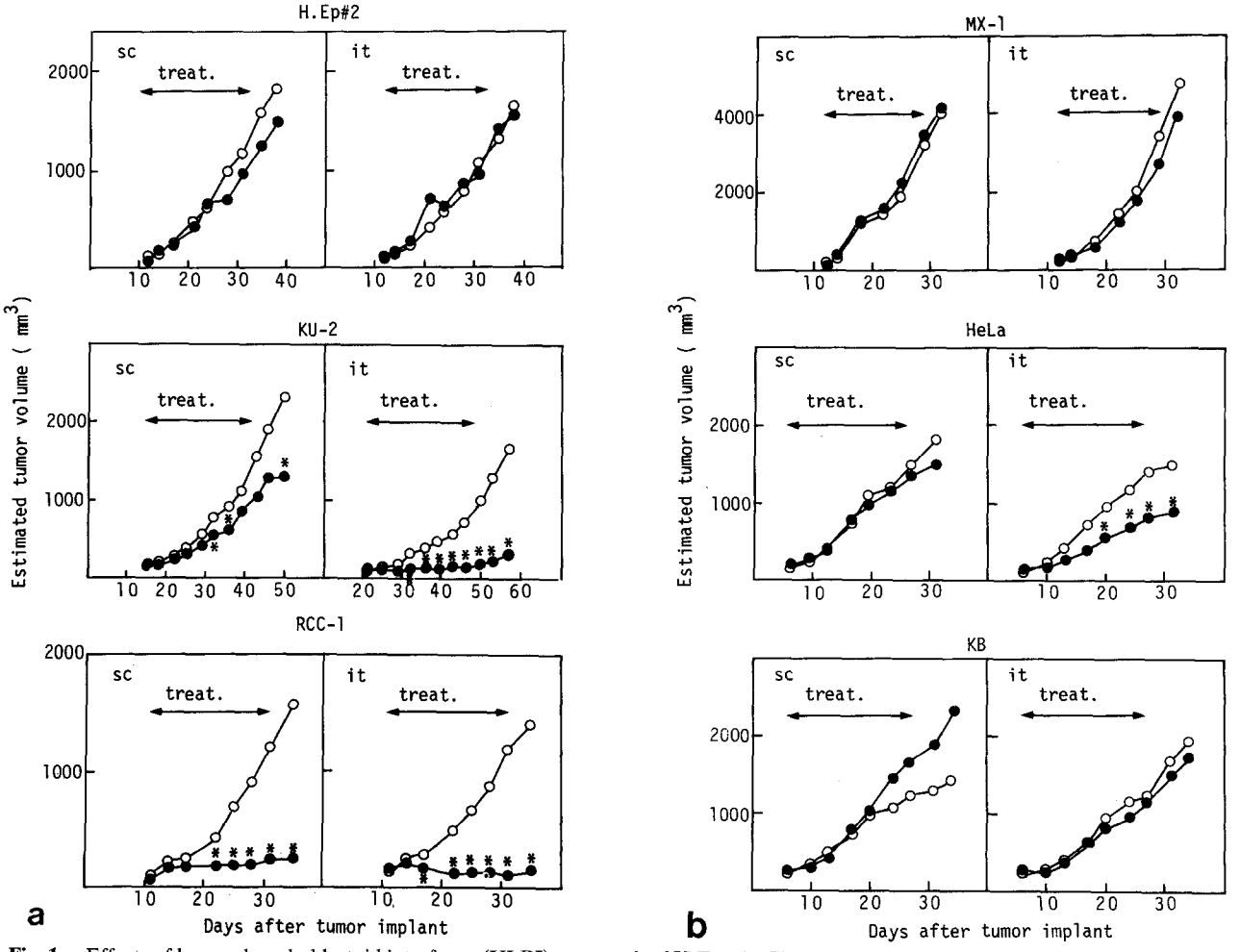

Fig. 1. a Effects of human lymphoblastoid interferon (HLBI) on growth of H.Ep#2, KU-2, and RCC-1 in nude mice. *SC,* subcutaneous injection; *IT,* intratumoral injection; *open circle,* vehicle; *closed circle,* HLBI (10^5 IU/mouse/day); $^*P < 0.05$. Each *point* represents mean estimated tumor volume for six to nine mice. **b** Effects of human lymphoblastoid interferon (HLBI) on growth of MX-1, HeLa, and KB in nude mice. *SC,* subcutaneous injection; *IT,* intratumoral injection; *open circle,* vehicle; *closed circle,* HLBI (10^5 IU/mouse/day); $^*P < 0.05$. Each *point* represents mean estimated tumor volume for six to nine mice

Results

The growth curves of six human tumors implanted in nude mice, which were then treated with vehicle and HLBI, are shown in Fig. 1A and B. HLBI was given to nude mice at a dose of 10^5 IU/mouse in daily SC or IT injections for 2–4 weeks.

KB, H.Ep#2, and MX-1 did not respond at all to HLBI therapy. The growth of HeLa was suppressed to some extent by IT injection of HLBI, but not by SC injection. In contrast, the growth of two renal cell carcinomas, KU-2 and RCC-1, was inhibited not only by IT injection, but also by SC injection.

The growth inhibiton of KU-2 by SC injection of HLBI was statistically significant ($P < 0.05$) on days 32, 36, and 50 after tumor implantation. The mean tumor weights measured on the 7th day after final administration of HLBI were 1.85 g in the control group and 1.27 g in the treated group. On the other hand, IT injection markedly increased the growth-inhibitory effects of HLBI on KU-2. Only a gradual increase of the estimated tumor volume was observed in the treated group. This effect was also shown clearly by the mean tumor weights, which were 1.18 g in the control group and 0.16 g in the treated group. In addition, when HLBI was given to nude mice at a dose of 10^6 IU/mouse in daily SC injections, the more apparent growth inhibition of KU-2 was observed without any weight losses, as shown in Figs. 2 and 3.

As shown in Fig. 1A, HLBI exerted the most pronounced growth-inhibitory effect on RCC-1. Both SC and IT injections of HLBI almost completely inhibited the tumor growth after a lag period of 2–5 days. Following SC injection, mean tumor weights were 0.53 g in the control group and 0.08 g in the treated group. Following IT injection, the corresponding weights were 0.56 g and 0.03 g, respectively.

To determine the range of serum HLBI levels during the HLBI therapy described above, the pharmacokinetics of HLBI in nude mice was investigated. The results are shown in Fig. 4. In the single-injection group, HLBI was given to nude mice at a dose of 10^5 IU/mouse SC, and the serum levels were assayed. The serum HLBI levels reached a peak level of 4,390 U/ml 1 h later and subsequently declined in the first order fashion with a half-life of 4 h to 128 U/ml 24 h later. In the consecutive-injection group, SC injections of HLBI were given daily at a dose of 10^5 IU/mouse for 10 days, and the serum levels were assayed after the final injection. The time-course of serum HLBI levels in the consecutive-injection group was almost the same as that

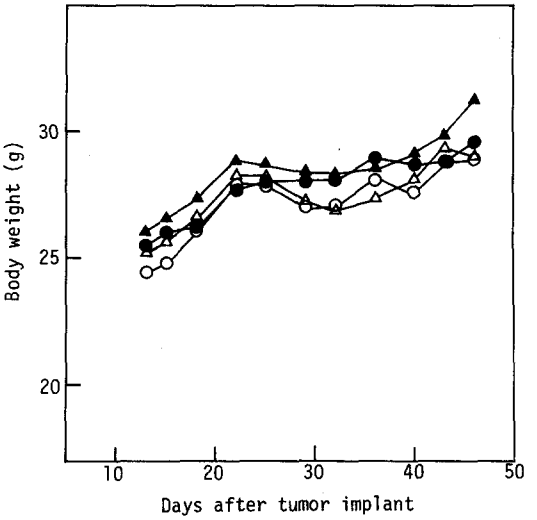

Fig. 3. Effect of human lymphoblastoid interferon (HLBI) on body weight of nude mice. HLBI was injected to nude mice SC at daily doses of 10^4, 10^5, and 10^6 IU/mouse for 3 weeks. *Open circle,* vehicle; *closed circle,* HLBI (10^4 IU/mouse/day); *open triangle,* HLBI (10^5 IU/mouse/day), *closed triangle,* HLBI (10^6 IU/mouse/day). Each *point* represents the mean body weight of six mice

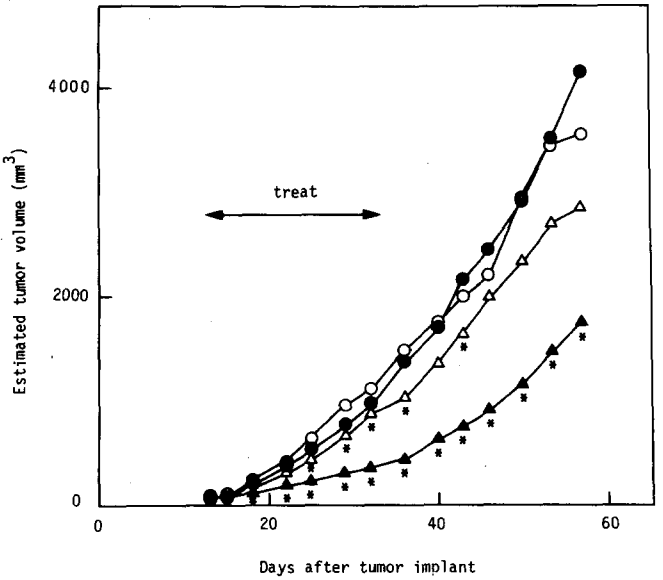

Fig. 2. Dose-response relationship for human lymphoblastoid interferon (HLBI) in growth inhibition of KU-2 implanted in nude mice. HLBI was injected to nude mice SC at daily doses of 10^4, 10^5, and 10^6 IU/mouse for 3 weeks. *Open circle,* vehicle; *closed circle,* HLBI (10^4 IU/mouse/day); *open triangle,* HLBI (10^5 IU/mouse/day); *closed triangle,* HLBI (10^6 IU/mouse/day); $^*P < 0.05$. Each *point* represents mean estimated tumor volume for six mice

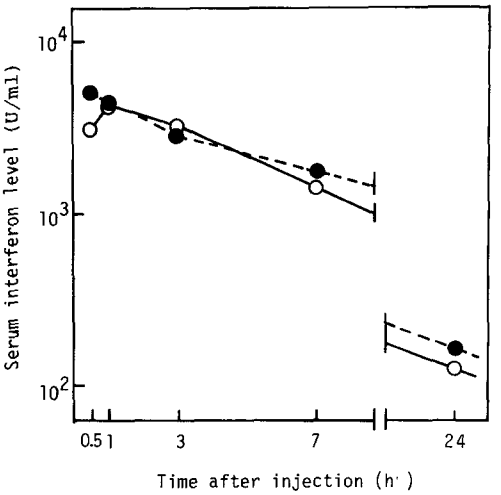

Fig. 4. Time course of serum human lymphoblastoid interferon (HLBI) levels in nude mice. *Open circle,* single SC injection of HLBI at a dose of 10^5 IU/mouse; *closed circle,* 10 consecutive SC injections of HLBI at a daily dose of 10^5 IU/mouse. In the case of 10 consecutive daily treatment, serum was obtained in each case after the final injection. Each *point* represents the mean value of serum HLBI levels of two mice

in the single-injection group. These observations show that HLBI did not accumulate significantly in the serum of nude mice and was not rapidly eliminated by antibody formation. Anti-interferon antibody does not appear to be produced by multiple injections of HLBI at this dose level in nude mice.

Discussion

In the present study, the effects of HLBI on six human tumor xenografts in nude mice were examined following SC or IT injections at daily doses of 10^5 IU/mouse for 2–4 weeks. HLBI markedly inhibited the growth of two renal cell carcinomas, KU-2 and RCC-1, with either injection route, while it exerted only a slight effect or none at all on the other tumors examined, MX-1, KB, HeLa, and H.Ep#2, even when given by IT injection. These results suggest that the antitumor effects of HLBI depend on the types of human tumors.

In recent clinical trials, renal cell carcinoma has been noted as one of the malignant tumors that is sensitive to α-type interferons [4, 7]. These clinical data may be relevant to the finding that both the renal tumors tested were retarded in their growth by HLBI in nude mice. Further investigations concerning the HLBI regimen and its combination with other anticancer drugs against renal cell carcinomas transplanted in nude mice are now being undertaken.

To elucidate the mode of in vivo action of HLBI, the relationship between its in vitro and in vivo antitumor activities was discussed. The four human tumors, KU-2, HeLa, KB, and H.Ep#2, used in this study can also be cultured in vitro. The in vitro anticellular effects of HLBI against these tumors have been determined in our laboratory as follows (T. Koide, H. Nakajima, and I. Sugata, personal communication): The 50% inhibitory concentrations (IC_{50}) were 90 IU/ml for Ku-2, 20,000 IU/ml for HeLa, 8,000 IU/ml for KB, and 20,000 IU/ml for H.Ep#2. The growth of KU-2 transplanted in nude mice was inhibited by both SC and IT injections at a daily dose of 10^5 IU/mouse, and particularly, almost completely inhibited by IT injection, as expected from its in vitro sensitivity to HLBI. IT injection of HLBI inhibited the growth of HeLa to some extent, while it did not suppress that of KB or H.Ep#2 at all. These results are not consistent with the tumors' in vitro sensitivity to HLBI, indicating that factors other than direct anticellular action may be involved in the in vivo effects of HLBI.

When HLBI was given SC to nude mice at a dose of 10^5 IU/mouse, its serum levels reached a peak level of 4,390 U/ml 1 h later and declined with a half-life of 4 h to 128 U/ml 24 h later. This pattern of HLBI serum levels was hardly affected by ten consecutive daily injections of HLBI, suggesting that the consecutive administration of HLBI to nude mice induces neither its rapid elimination by means of antibody formation nor accumulation at a dose level of 10^5 IU/mouse. The serum HLBI levels, therefore, are considered to be retained between about 5,000 U/ml and 100 U/ml during HLBI therapy. This range of HLBI serum concentrations was more than the IC_{50} for KU-2, while less than the IC_{50} for HeLa, KB, and H.Ep#2. Since SC injection of HLBI was only effective for KU-2, the in vivo antitumor effect of HLBI is considered to be a direct anticellular action against tumor cells. This is also supported by the fact that IT injection was more effective than SC injection for KU-2 and HeLa, because the former resulted in 30-fold higher levels of HLBI in tumor tissues than the latter at 3 h after injection (T. Yamaoka and Y. Yanagi, unpublished data).

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