Pharmacokinetics and Antitumor Effects of an Interleukin-2 Immunocomplexing Agent in Murine Renal Cell Carcinoma

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Background: Conventional therapy for renal cell carcinoma (RCC) using systemic administration of interleukin-2 (IL-2) has shown limited anti-tumor action. The purpose of this study was to investigate the anti-tumor effects of a newly developed immune complex of IL-2 (IC) against RCC.

Methods: IC was prepared by mixing IL-2 and an anti-IL-2 monoclonal antibody at a molar ratio of 2:1. The pharmacokinetics and anti-tumor effects of IC were then studied in a murine RCC line, Renca.

Results: Serum IL-2 levels were sustained longer in mice given IC than in mice given IL-2 alone after either subcutaneous or intratumoral injections. After an intratumoral injection of IC, the IL-2 concentration in the tumor nodules remained higher compared with mice given IL-2 alone. The anti-tumor effect was most pronounced in mice treated with intratumoral injections of IC.

Conclusions: Results obtained here indicate that an immune complex of IL-2 provides a useful tool for the treatment of RCC by altering the pharmacokinetics of IL-2 in vivo.

INTRODUCTION

The prognosis of patients with renal cell carcinoma (RCC) is poor once the disease recurs either locally, or has become metastatic. This is due to the fact that RCC is extremely insensitive to either radiotherapy or anti-cancer chemotherapy. These features have provided the impetus for continued study of immunotherapy for RCC.

Interleukin-2 (IL-2), originally discovered as a T-cell growth factor, is a lymphokine produced by helper T lymphocytes. Treatment of tumor-bearing mice or patients with IL-2 alone, or combined with the adoptive transfer of lymphocytes activated by IL-2, has been shown to induce regression of RCC. However, conventional systemic IL-2 therapy has shown only limited anti-tumor activity. Additionally, there are problems associated with the clinical use of IL-2, including the fact that IL-2 is rapidly cleared from the blood. In order to increase the effectiveness of IL-2 in vivo, it is necessary for the IL-2 to act directly on the tumor cells for a longer period of time and in a higher concentration. Therefore, we considered administration of IL-2 directly to the tumor site using a newly developed immune complex of IL-2. The immune complex was prepared by mixing IL-2 with an anti-IL-2 monoclonal antibody, and was predicted to augment the anti-tumor effect by altering the in vivo distribution of IL-2 as the complexed IL-2 has a greater molecular weight compared to that of IL-2 alone. This study was undertaken to investigate the anti-tumor effects and biological properties of an immune complex of IL-2 in mice bearing a murine renal cell carcinoma.

MATERIALS AND METHODS

Animals
Female BALB/c mice were purchased from Charles River Japan (Japan). The animals were kept in an established pathogen-free conditioned environment and fed a commercial pellet diet CE-2 (CLEA, Japan). Six-week-old mice were used for all experiments.

Tumor Cells
Renca, a transplantable murine RCC of spontaneous origin, was established at the National Cancer Institute (Bethesda, MD, USA).

Tumor Transplantation
The masses of Renca tumors were excised and cut into about 3 mm x 3 mm pieces. Each fragment was then implanted subcutaneously into the back of a mouse using a trocar needle.

Interleukin-2
Recombinant human IL-2 used in these experiments was produced by DDS Research Laboratories, Takeda Chemical Industries (Osaka, Japan).
Immune Complex

An immune complex consisting of IL-2 and a monoclonal antibody (MoAb) against IL-2 was prepared by Takeda Chemical Industries. Briefly, 0.2 mg of IL-2 was mixed with 1.0 mg of MoAb-HRL1-52 (molar ratio 2:1) as previously described, in 1.2 mL of phosphate-buffered saline (PBS) and the reaction mixture was incubated at room temperature for 10 minutes. MoAb-HRL1-52 has been shown not to neutralize the biological properties of IL-2 in this immune complex formation.

IL-2 Determination

IL-2 levels in the serum and tumor tissue were measured by an enzyme-linked immunosorbent assay (ELISA). When tumor nodules grew to 12-14 mm in diameter at 18 days after implantation of the tumor, the mice were divided into two groups according to the site of administration, either via subcutaneous injection or by intratumoral injection. A dose of 10 μg as IL-2 was given per injection to the mice bearing the Renca tumors. Blood was taken from the retroorbital sinus, and tumor tissue was taken surgically at various times after administration of IL-2 or its immune complex. The tumors were homogenized in PBS, and then subjected to an ELISA for the determination of IL-2. The concentration of IL-2 was given as the average level from two mice.

Experimental Model

When tumor nodules grew to 6-8 mm in diameter at 14 days after implantation, the mice were separated into groups consisting of six mice each and therapy was started. IL-2 or the immune complex was dissolved in PBS and a 0.1 mL suspension of the solution was administered by subcutaneous injection at sites distant from the tumor or by intratumoral injection at a dose of 1 μg or 10 μg as IL-2 daily for 10 days. The perpendicular diameters of the tumors were measured with a sliding caliper and the tumor weights were estimated by the formula: (long diameter) × (short diameter) / 2. Statistical analyses were carried out based on growth ratios to tumor volumes before treatment in each group using the Student's t-test. Survival rates were calculated by the Kaplan-Meier method and the statistics determined by the Wilcoxon rank sum test.

Spleen Cell Cytotoxicity Assay

The effects of the administration of IL-2 or its immune complex on NK activity were also evaluated. Mice were sacrificed by cervical dislocation 10 days after receiving daily subcutaneous injections of IL-2 or the immune complex at a dose of 10 μg as IL-2, and the spleens were removed. The cell suspension was layered over a density separation medium (Lympholyte-M, Cedarlane, Lab, Canada) to isolate the spleen cells. Cytotoxicity was assayed in triplicate with a 4-hour 51Cr-release assay. The target cells used for these experiments were YAC-1, a murine lymphoma cell line. YAC-1 cells were labeled with 100 μCi of Na11CrO4 (Dai-ichi Radioisotope Lab., Japan). The effector cells (murine spleen) were used at an effector/target (E/T) ratio of 10:1 or 40:1. All cytotoxicities were expressed as the average release of chromium from three mice.

RESULTS

Pharmacokinetics of IL-2 and the Immune Complex

Following subcutaneous or intratumoral injection of IL-2 or its immune complex at doses which represented 10 μg as IL-2, IL-2 concentrations in the serum and tumor tissue were determined by ELISA at various times after administration. Injection of the immune complex resulted in a higher serum IL-2 concentration for up to 10 hours after in vivo administration compared with administration of IL-2 alone, in both the subcutaneous and intratumoral injection groups. IL-2 concentrations in the tumor tissue were much higher in mice treated by intratumoral injection than in mice treated by subcutaneous injection using either the immune complex or IL-2 alone. After an intratumoral injection of the immune complex, IL-2 concentrations in the tumor nodule remained higher up to three hours after administration compared with administration of IL-2 alone. After a subcutaneous injection of the immune complex, the mice showed higher IL-2 concentrations in the tumor tissue, with a maximum level of 25.75 ng/g IL-2 injected at three hours (Table 1).

Table 1. Concentration of IL-2 in serum and tumor tissue after the administration of IL-2 or an immune complex of IL-2 at a dose of 10 μg as IL-2 either by a subcutaneous or intratumoral injection.

| Treatment | Injection | IL-2 Serum | Immune complex Serum |
|-----------|-----------|------------|----------------------|
| Hours after administration | ng/mL | ng/mL | ng/mL | ng/mL |
| 1 | 149.10 | 32.13 | 146.60 | 21.15 |
| 3 | 14.47 | 4.87 | 15.64 | 15.03 |
| 6 | 0.72 | 1.58 | 17.00 | 7.50 |
| 10 | 0.29 | 0.16 | 0.61 | 0.90 |
| 24 | 0.27 | 0.21 | 0.19 | 0.28 |

Table 1.

For sc, subcutaneous injection; it, intratumoral injection.
Antitumor Activity

The effects of IL-2 and the immune complex on the growth of RCC tumors are shown in Table 2. Neither IL-2 or the immune complex injected at a dose of 1 μg of IL-2 inhibited tumor growth by either route of injection. On the other hand, administration of IL-2 or the immune complex at a dose of 10 μg as IL-2, by either injection route, significantly inhibited the growth of Renca tumors. The anti-tumor effect was most pronounced in the mice treated with an intratumoral injection of the IL-2 immune complex. Tumors disappeared in 2 of 6 mice treated by a daily intratumoral injection of immune complex at a dose of 10 μg as IL-2. The survival rate was significantly (*P < 0.05) extended in the group treated with an intratumoral injection of the immune complex at a dose of 10 μg as IL-2 (Fig. 1).

Table 2. Effect of IL-2 and an immune complex of IL-2 on the growth of Renca murine renal cell carcinoma.

| Treatment* | Tumor weight† |
|------------|---------------|
|            | Before treatment | 2 days | 6 days | 10 days |
| No treatment | 129 ± 2.5 | 469 ± 140 | 1327 ± 174 | 2249 ± 193 |
| PBS 1 μg q.i.d | 114 ± 19 | 476 ± 65 | 1589 ± 458 | 2377 ± 440 |
| IL-2 1 μg q.i.d | 132 ± 9 | 430 ± 77 | 1157 ± 159 | 1835 ± 267 |
| IL-2 1 μg q.d | 141 ± 16 | 171 ± 79 | 1186 ± 150 | 2012 ± 147 |
| IC 1 μg q.i.d | 125 ± 17 | 419 ± 106 | 1167 ± 151 | 214 ± 294 |
| IC 1 μg q.d | 128 ± 15 | 181 ± 112 | 1059 ± 196 | 1881 ± 135 |
| IL-2 10 μg q.i.d | 113 ± 20 | 100 ± 106 | 942 ± 224 | 1427 ± 112 |
| IL-2 10 μg q.d | 129 ± 12 | 243 ± 61 | 924 ± 271 | 1287 ± 106 |
| IC 10 μg q.i.d | 128 ± 21 | 280 ± 64 | 936 ± 115 | 1482 ± 122 |
| IC 10 μg q.d | 146 ± 20 | 245 ± 51 | 560 ± 175 | 760 ± 289 |

* Renca tumors were implanted subcutaneously into BALB/c mice on day 0. Therapy was then given beginning on day 14 by daily injection of IL-2 or the immune complex for 10 days. † Mean tumor weight ± standard deviation. ‡ P < 0.05, when compared with values of untreated mice. § P < 0.01, when compared with values of untreated mice. PBS, phosphate-buffered saline; IC, immune complex; sc, subcutaneous injection; it, intratumoral injection.

Fig. 1. Survival rates of BALB/c mice after initiation of treatment, calculated by the Kaplan-Meier method. IC, immune complex; sc, subcutaneous injection; it, intratumoral injection. - - - Control; - - - IC, it; - - - - IC, sc; - - - - IL-2, it; - - - - IL-2, sc.

Table 3. Effect of IL-2 and an immune complex of IL-2 on natural killer activity in mice.

| E/T ratio | Control | IL-2 | IC | IL-2 | IC |
|-----------|---------|------|----|------|----|
| 1/0/1     | 4.1     | 12.4 | 20.3| 11.6 | 18.5|
| 4/0/1     | 9.6     | 15.9 | 25.7| 11.1 | 21.0|

Cell-mediated cytotoxicities of splenocytes were evaluated after daily subcutaneous or intratumoral injections of IL-2 or an immune complex at a dose of 10 μg as IL-2 for 10 days. The control group received subcutaneous injections of phosphate-buffered saline. The cytotoxicities were expressed as a mean value from three mice. E/T ratio, effector/target cell ratio; IC, immune complex; sc, subcutaneous injection; it, intratumoral injection.

NK Activity

Mouse spleen cells were harvested after 10 daily injections of IL-2 or the immune complex at a dose of 10 μg as IL-2 and served as the effector cells in the cytotoxicity assay. Cytotoxicity against YAC-1 cells was augmented by the administration of either IL-2 or the immune complex. The mice treated with the immune complex demonstrated greater cytotoxicity compared to mice treated with IL-2 alone. There were no marked differences between mice treated by subcutaneous injection or mice treated by an intratumoral route (Table 3).

DISCUSSION

The pharmacokinetics of IL-2 and its effects on the growth of tumors implanted into mice were studied in order to investigate the potential clinical application of an immune complex of IL-2 in the treatment of advanced RCC. The anti-tumor activity of the immune complex was estimated using the murine renal cell carcinoma line, Renca, which is widely used as an animal model for the immunotherapy of RCC.9,10

One of the problems associated with the clinical use of IL-2 is its short half life. In addition, IL-2 has a large distribution to multiple tissues or organs, which results in low plasma levels, and consequently causes severe adverse effects in these tissues and organs. On the other hand, murine immunoglobulin G molecules are known to have a small distribution volume and a long plasma half life.9,10 It has already been reported that conjugation of IL-2 with murine immunoglobulin G or other macromolecular compounds prolongs the plasma half life of IL-2 and enhances the anti-tumor activity of IL-2.11,12 We hypothesized that an immune complex of IL-2 would effectively produce immune activation if administered directly to the tumor site, expecting a depot effect due to the larger molecular weight of the conjugate of IL-2 similar to that using immunoglobulin.
The anti-tumor effects of IL-2 and the immune complex were effective in a dose-dependent manner, whereby both IL-2 and the immune complex at a dose of 10 μg significantly inhibited growth of the tumor by either subcutaneous or intratumoral injections. This anti-tumor effect was most pronounced in the group treated with an intratumoral injection of the immune complex.

Results from the pharmacokinetic studies showed that after subcutaneous administration of the immune complex, a higher serum IL-2 concentration was sustained in these mice up to ten days after administration as opposed to mice treated with IL-2 alone. However, tumor growth inhibition was most effective in mice treated by an intratumoral injection of the immune complex. The results also show that IL-2 concentrations in murine tumor tissue treated by a subcutaneous injection of the immune complex were lower than in mice treated by an intratumoral injection of the immune complex, suggesting that the best distribution of IL-2 to the target site was achieved by an intratumoral injection of the immune complex. The sustained serum concentration of IL-2 by treatment with the immune complex also efficiently activated NK activity in the spleen cells.

From a clinical standpoint, there may be additional advantages for the use of the immune complex since a higher serum level of IL-2 can be maintained by the administration of a lower dose of immune complex as compared with the administration of IL-2 alone. These results also suggest that the use of an IL-2 immune complex may decrease the adverse systemic effects of IL-2.

Utilizing an immune complex of IL-2 produced by the DDS Research Labs., we have successfully treated a murine renal cell carcinoma transplanted into mice. The results indicate that the immune complex may be effective in the treatment of RCC as a sustained release preparation by subcutaneous or intratumoral injection. Clinical treatments may include such options as ultrasound-guided injection to treat local or metastatic recurrent lesions, transcatheter arterial infusions in patients with unresectable primary lesions in the kidney or hepatic and skeletal metastatic lesions, or peritumor injection by bronchoscopy to treat metastatic lung lesions.

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