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Improved Anti-tumor Activity and Safety of Interleukin-13 Receptor Targeted Cytotoxin by Systemic Continuous Administration in Head and Neck Cancer Xenograft Model

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Abstract

Background: IL-13 receptor (IL-13R) targeted cytotoxin, IL13-PE38QQR, has been shown to have very potent anti-tumor activity to IL-13R-expressing head and neck tumor cells in vitro and in vivo. However, its effect is limited in aggressive tumors. To further improve the anti-tumor activity and safety of IL-13 cytotoxin, we employed continuous infusion technique in animal model of head and neck cancer.
Materials and Methods: We surgically implanted continuous infusion (CI) pump intraperitoneally that released drug for 7 days, and its anti-tumor effect was evaluated. A comparison was made for antitumor activity and safety with intravenously (IV) administered IL-13 cytotoxin in a head and neck (KCCT873 and HN12) subcutaneous (SC) xenograft tumor models in nude mice. Vital organ toxicities were assessed by histologic examinations and blood serum chemistry analyses.
Results: The 50 or 75 μg/kg/day for 7 days of IL-13 cytotoxin either by IV or CI administration did not show any difference in safety or anti-tumor activity. IV administration of 150 or 200 μg/kg/day of IL-13 cytotoxin for 7 days was lethal to nude mice, whereas 200 μg/kg/day $\times$ 7 days of CI administration was highly effective in the regression of established tumors without any toxicities. Additionally, CI administration of IL-13 cytotoxin (200 μg/kg/day) showed growth inhibition of larger HN12 tumors in nude mice.
Conclusion: With a CI schedule, IL-13 cytotoxin can be systemically administrated at approximately twice the dose otherwise given by daily IV bolus administration.

Introduction

Desirable anti-tumor activities and unexpected toxicities of novel anti-tumor agents depend partly on drug delivery routes. Even a potentially powerful anti-tumor agent can cause unexpected serious adverse events or organ toxicities by a certain route of drug administration. To resolve these problems, therapeutic approaches such as prolonged drug release mechanisms or continuous infusion of drug have been reported to increase drug efficacy (1–4). Because of shorter half-lives of drugs, intravenous (IV) or intraperitoneal (IP) administration limits their efficacy. Moreover, multiple bolus administrations increase the susceptibility to organ toxicities. Therefore, continuous infusion (CI) of drugs can be an ideal way of systemic administration of novel cancer therapeutics.

Interleukin-13 (IL-13) is a helper T cell type 2 (Th2)-derived pleiotropic immune regulatory cytokine (5). It has predominant biological activities on B cells, monocytes, fibroblasts, and endothelial cells and plays a major role in inflammatory diseases. IL-13 may also play a prominent role in cancer because receptors for this cytokine are overexpressed. IL-13 is also an autocrine growth factor for some cancer cells (6). We first identified plasma membrane receptors for IL-13 on several human renal cell carcinoma cell lines (7,8), and since then we reported that a variety of human solid cancer cell lines including AIDS-associated Kaposi's sarcoma (9,10), glioblastoma (11,12), prostate cancer (13), ovarian carcinoma (14), and head and neck cancer (SCCHN) (15–17) express receptor for IL-13 (IL-13R). In recent years, the receptors for IL-13R have been extensively characterized. We have demonstrated that IL-13R may exist as three different forms in different cell types (7,14,18–21). Two different chains of the IL-13R, IL-13Rα1 (also known as IL-13Rα') and IL-13Rα2 (also known as IL-13Rα) have been cloned. The murine and human IL-13Rα1 chain was cloned first (22–24). This chain binds IL-13 with low affinity but when coupled with IL-4Rα chain (also known as IL-4Rβ) binds IL-13 with high affinity and mediates IL-13–induced signaling (14,19,20,23,25). The second chain of IL-13R, IL-13Rα2, was cloned from a human renal cell carcinoma cell line (Caki-1). This chain has 50% homology to IL-5R at the DNA level, has a short intracellular domain, and binds IL-13 with

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approximately 50-times higher affinity than IL-13Rα1 chain (26,27). More recently, we have reported that IL-13Rα2 chain can play an important role in receptor binding and internalization (28,29).

Based on our findings that many solid cancer cells express IL-13R, we produced IL-13 cytotoxin, termed IL13-PE38QQR, which is composed of IL-13 and a mutated form of a *Pseudomonas* exotoxin. IL13-PE38QQR has a potent anti-tumor activity to IL-13R expressing tumor cells in vitro (8,9,13,15,17,30,31) and in vivo (10,16,32,33). Despite the success of preclinical animal studies, the effect of systemic injection of IL-13 cytotoxin has been limited in some of the most aggressively growing head and neck tumor xenograft models (16). To achieve the optimum effect of this targeted agent, a higher dose needs to be administrated. However, systemic bolus injection of higher doses caused organ toxicities as described previously (10,33). The major organ damage caused by bacterial toxin is irreversible liver toxicity (34–36). To avoid liver toxicity, systemic drug exposure needs to be either of a lower dose or prolonged. Thus, we hypothesized that continuous release of IL-13 cytotoxin in the systemic circulation would enhance its anti-tumor effect as well as reduce organ toxicities. Therefore, in this study we employed mini-osmotic pumps that can infuse drugs continuously and compared the anti-tumor activity and safety of IL-13 cytotoxin given by IV bolus injections and by CI.

Materials and Methods

Recombinant Cytotoxin and Cell Lines

Recombinant IL13-PE38QQR was produced and purified in our laboratory (17,30). The purified protein was found to have 3200 endotoxin EU/mg protein. The final concentration of endotoxin injected to animals ranged between 3.2 and 4.8 EU/dose. The range of endotoxin is lower than the allowable limit in the clinic. Human head and neck cancer cell line WSU-HN12 (termed HN12) was a kind gift from Dr. Andrew Yeudall (National Dental and Craniofacial Research Institute, NIH, Bethesda, MD, USA) (37). KCCT873 cell line was established at Research Institute, Kanagawa Cancer Center (Yokohama, Japan) (38). Cells were cultured in Eagle's Modified Essential Medium (HN12) or RPMI 1640 (KCCT873) containing 10% fetal bovine serum (Biowhittaker Inc., Walkersville, MD, USA), 1 mM HEPES, 1 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Biowhittaker).

Animal Studies

Athymic nude mice 4 weeks old (about 20 g in body weight) were obtained from the Frederick Cancer Center Animal Facilities (National Cancer Institute, Frederick, MD, USA). Animal care was taken in accordance with the guidelines of the NIH Animal Research Advisory Committee. Human head and neck tumor xenografts were established in the nude mice by subcutaneous (SC) injection of cells into the flank. HN12 or KCCT873 cells (5×10^6) were injected in 150 μl of PBS. Palpable tumors developed within 3–4 days. The mice then received injections of excipient (0.2% HSA in PBS) or chimeric toxin either IV (150 μl using a 27-gauge needle through the tail vein) or CI (0.5 μl/hr for 7 days). Continuous administration was performed by loading a mini-osmotic Alzet pump (Alza, Palo Alto, CA, USA) with 100 μl IL-13 cytotoxin. The pump was surgically implanted IP on day 4 after tumor implantation. In brief, nude mice were anesthetized with ketamine and xylazine and placed in the supine position. An upper midline abdominal incision was made, and pumps were inserted from the top of the device.

Statistical Analysis

Two perpendicular diameters of tumors were carefully measured by a Vernier caliper and tumor size was then calculated by multiplying the length and width of the tumor on a given day. The statistical significance of tumor regression was calculated by Student's *t* test.

Histologic Analysis

Organs from the experimental animals were fixed in 10% formalin and 5-μm tissue sections were prepared, and stained with hematoxylin and eosin.

Results

A Better Anti-tumor Activity of IL13-PE38QQR by CI Compared with IV Administration in KCCT873 Tumor Xenografts

To compare the anti-tumor activity of IL-13 cytotoxin via IV administration and CI, 50 or 75 μg/kg/day of IL-13 cytotoxin was injected IV (one injection per day for 7 days) or mini osmotic pumps (350 or 525 μg/kg total infusion for 7 days) were surgically implanted IP on day 4 in KCCT873 tumor xenografts. As shown in Figure 1 (A and B), KCCT873 tumors without treatment (excipient control) grew well and mean tumor size became 162–190 mm^2 by day 31. On the other hand, tumors in the treated mice began regressing during the treatment period; however, when the treatment period was over, all the tumors began growing gradually. Although in mice treated with 50 μg/kg/day of IL-13 cytotoxin by both routes showed significant regression of tumor in size (79 mm^2 in IV and 66 mm^2 in CI tumors) by day 31 ($p < 0.008$ compared to control), there was no significant difference ($p = 0.14$) in the anti-tumor effect between IV and CI drug administration groups.

In mice treated with 75 μg/kg/day of IL-13 cytotoxin (Fig. 1B), all tumors began to grow slowly after the treatment period. However, the anti-tumor activity of IL-13 cytotoxin by CI was slightly better compared with IV administration, although the difference was not statistically significant. Mice

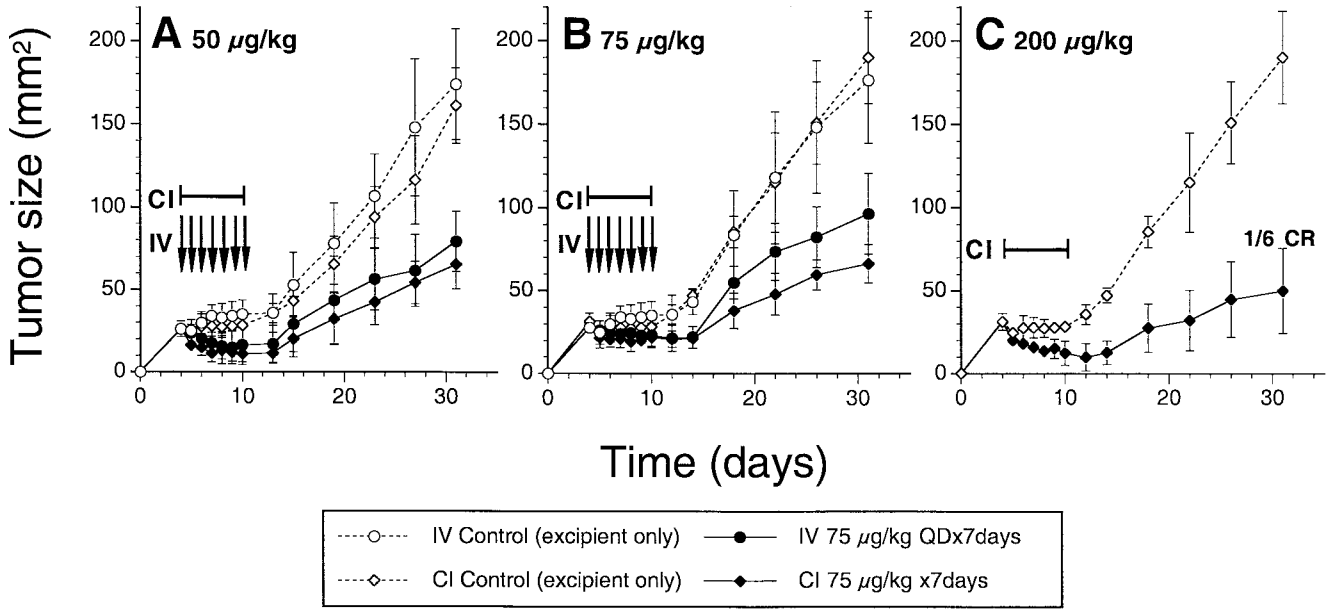

Fig. 1. Comparison of anti-tumor activity of IL-13 cytotoxin in KCCT873 tumor xenografted nude mice between IV and CI routes. Male mice bearing subcutaneous tumor were treated with 50 μg/kg/day **(A)**, 75 μg/kg/day **(B)**, or 200 μg/kg/day **(C)** of IL13-PE38QQR by either IV or continuous infusion (CI) from day 4–10 (7 days). *Abbreviations:* CR, complete response. Experiments were performed twice with similar results.

were also given 200 μg/kg/day of IL-13 toxin by CI (total 1400 μg/kg during 7 days). Two out of six tumors completely disappeared by day 12. Although one tumor recurred, by day 31 one mouse remained tumor free and the mean size of tumors was significantly smaller (50 mm^2) compared with tumors in mice with excipient control-loaded pump (190 mm^2) ($p < 0.001$) (Fig. 1C).

Evaluation of Systemic Administration of IL13-PE38QQR in HN12 Tumor Xenografts

We next examined the maximum tolerable dose of IL-13 cytotoxin by systemic administration of the drug. Four days after the implantation of HN12 tumors in nude mice, animals were treated by either IV (150 or 200 μg/kg/day for 7 days; total 1050 or 1400 μg/kg, respectively) or CI (300 μg/kg/day for 7 days; total 2100 μg/kg). As shown in Figure 2A, all the treated tumors started regressing as soon as the treatment began. However, in the 200 μg/kg/day IV treatment group ($n = 4$), all the mice died by day 11. In the 150 μg/kg/day IV treatment group ($n = 8$), seven out of eight mice died by day 12. The last mouse left was sick during the treatment period, but recovered later. The survival rate from toxicity in both IV treatment groups was poor (12.5% in 150 μg/kg and 0% in 200 μg/kg) (Fig. 2B).

In sharp contrast, all mice in the 300 μg/kg/day CI treatment group ($n = 8$) remained healthy throughout the experimental period. In two out of eight mice complete disappearance of tumors was observed by day 10 (Fig. 2A). Although tumors started to grow again, the mean size of the tumors on day 27 (24 mm^2) was significantly smaller ($p < 0.005$) than the mean size of control tumors (208 mm^2). The survival rate in this group was 100% compared to control group on day 30 (Fig. 2B).

To assess the organ toxicities after treatment with IL-13 cytotoxin by either IV or CI routes, blood serum chemistry and histologic analyses of vital organs were performed. Blood was drawn on day 11 (1 day after the completion of the treatment period) for serum chemistry. As shown in Table 1, after 150 μg/kg/day IV administration of IL-13 cytotoxin, blood serum potassium, CPK, LDH, and AST/ALT levels were greatly increased compared with blood samples from excipient only injected. Animals that received 200 or 300 μg/kg/day by CI also showed elevations in CPK, LDH, and AST/ALT; however, these increases were not as high as seen in IV treated mice.

Samples from vital organs such as kidney, liver, lung, and spleen were also harvested on day 10 or 11 and histology of the tissue sections was assessed. As summarized in Table 2, after 150 or 200 μg/kg/day for 7 days of IV administration of IL-13 cytotoxin, major histologic changes in vital organs were found. As expected, these histologic changes were more severe in the 200 μg/kg/day IV treated mice compared with the 150 μg/kg/day IV treated mice. Organ toxicities including notable liver necrosis, multifocal necrosis of kidney, and pulp degeneration of spleen were observed in 200 μg/kg/day for 7 days IV treated mice. Tissue sections from 100 μg/kg/day IV treated mice did not show significant histologic changes (data not shown). These histologic changes were considered to be signs of organ toxicities caused by IL-13 cytotoxin.

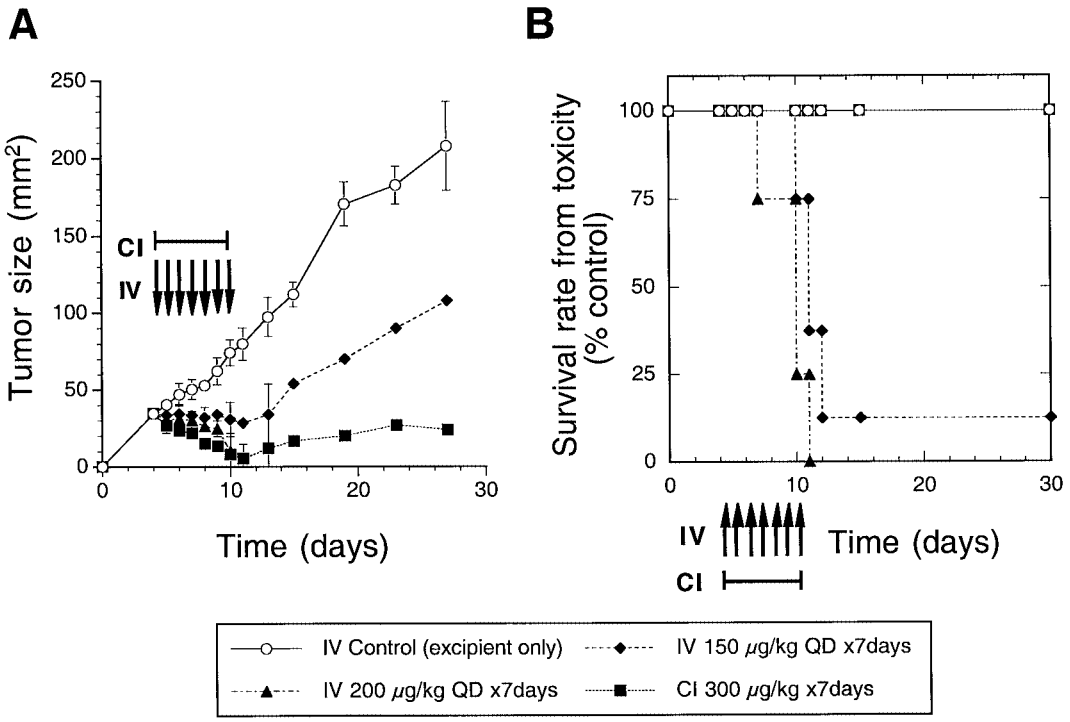

Fig. 2. Toxicity study in HN12 tumor xenografted nude mice. Male mice bearing subcutaneous tumors were treated with excipient only IV (O; $n = 6$), 150 μg/kg/day IV (◆; $n = 8$), 200 μg/kg/day IV (▲; $n = 4$), or 300 μg/kg/day CI (■; $n = 8$) of IL13-PE38QQR from day 4 to 10 (7 days). **(A)** Anti-tumor activity of IL-13 toxin is shown. Bars represent the SD. **(B)** survival rates are expressed as percentage of surviving mice compared with control. Experiments were performed twice with similar results.

On the other hand, the mice treated with 200 and 300 μg/kg/day of CI administration of IL-13 cytotoxin did not show severe histologic changes. Although slight abnormalities were found in liver and kidney samples from the 300 μg/kg/day CI treated mice, changes were apparently modest compared with 150 μg/kg/day IV treated mice. As shown in Figure 3, 200 μg/kg IV dose caused considerable histologic changes in vital organs. Considering 200 μg/kg/day dose delivered by either IV injections or CI were same in total dose (1400 μg/kg for 7 days), these results suggest that CI route is definitely less toxic to vital organs than IV route.

Continuous Infusion Increased Anti-tumor Activity of IL13-PE38QQR in HN12 Tumor Xenografts

Our blood serum chemistry analysis and histologic examinations of vital organs suggested that 100 μg/kg/day (IV) or 200 μg/kg/day (CI) IL-13

Table 1. Changes in blood serum chemistry after the treatment with IL13-PE38QQR

Profile	Reference Range (Units)	Intravenous Administration		Continuous Infusion		
		Control*	150 μg/kg	Control	200 μg/kg	300 μg/kg
Sodium	150–162 (mEq/l)	150	141	151	152	144
Potassium	3.8–6.8 (mEq/l)	6.0	20.1	5.7	5.8	16.3
CPK†	0–800 (U/l)	179	14414	60	471	6665
LDH	260–680 (U/l)	1083	5032	663	1353	2490
AST (GOT)	72–288 (U/l)	208	1482	70	125	565
ALT (GPT)	24–140 (U/l)	76	723	28	88	91
Albumin	2.6–4.6 (g/dl)	2.8	1.9	2.5	2.7	3.4

* Data represent the mean of blood samples from two animals from each group.

† CPK, creatinine phosphokinase.

Table 2. Histologic changes in vital organs after the treatment with IL13-PE38QQR*

	Intravenous Administration		Continuous Infusion	
	150 μg/kg	200 μg/kg	200 μg/kg	300 μg/kg
Liver	Mild multi-focal necrosis, hydrophilic degeneration of pericentral vein cells	Atrophic change, Severe multi-focal necrosis, collapse of structure	NC	Mild cell degeneration
Lung	NC†	Alveolar wall thickness	NC	NC
Kidney	Cell hypertrophy, mild focal necrosis, basement thickness	Multi-focal necrosis	NC	Cell hypertrophy
Spleen	Fibrosis	Pulp degeneration	NC	NC

* Data represent common findings from two animals in each group.
† NC, no remarkable change.

cytotoxin can be administrated systemically for 7 days without toxic side effects. To compare the anti-tumor effect of 100 μg/kg/day (IV) or 200 μg/kg/day (CI), HN12 cells were implanted SC into male or female nude mice. IL-13 cytotoxin was administrated for 7 days from day 4 to day 10. As shown in Figure 4A, tumors began regressing during IV treatment period in both male and female mice, and by day 10, tumors disappeared in one out of six mice in both male and female groups. Tumors started growing after the treatment period and by day 14 tumors recurred in all the mice. Although the mean size of tumors was significantly smaller (male, 71 mm^2; female, 91 mm^2) compared with excipient only injected control tumors (male, 184 mm^2; female, 191 mm^2) on the day of termination of the experiment (day 27; $p < 0.0005$). No complete responders to IL-13 cytotoxin were observed. No significant difference was observed in the anti-tumor activity of IL-13 cytotoxin in either male or female mice.

As shown in Figure 4B, tumors in 200 μg/kg/day CI treatment group also regressed during treatment period in both male and female mice; however, the tumors regressed more quickly than the IV treatment group (100 μg/kg/day; Fig. 4A). By day 11, tumors completely disappeared in three out of six male mice and two out of six female mice. In the rest of mice, tumors began growing gradually after the drug infusion period; recurrence was observed in one each male and female mouse. Nevertheless, two of six male mice and one of six female mice remained tumor-free until termination of the experiment (day 27). The mean size of tumors in treated mice (male, 46 mm^2; female, 72 mm^2) were significantly

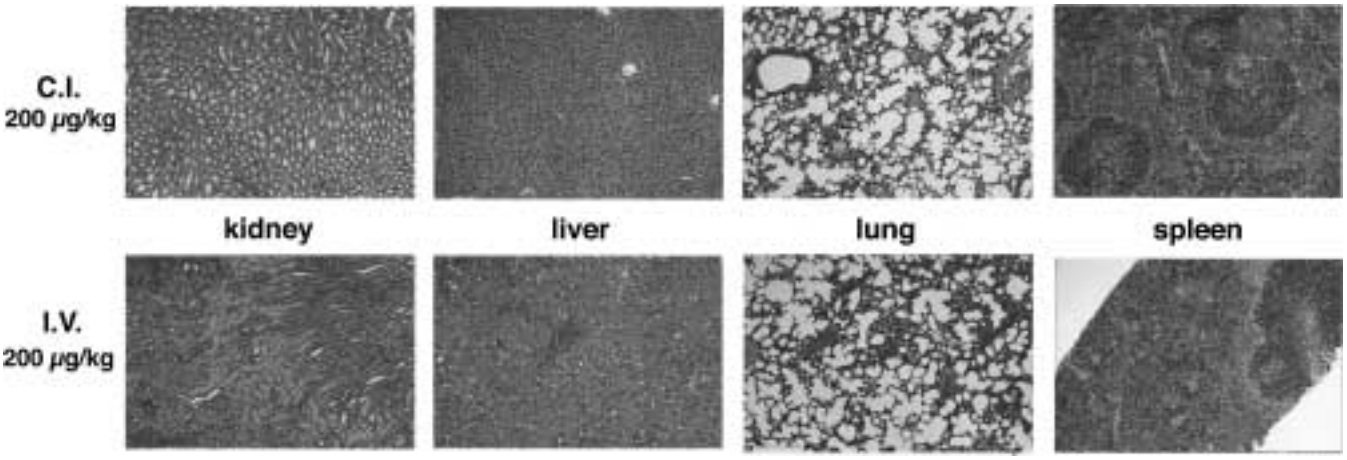

Fig. 3. Comparison of histologic changes after treatment with 200 μg/kg/day of IL-13 cytotoxin by IV or CI. Male mice bearing HN12 subcutaneous tumors were treated with 200 μg/kg/day of IL13-PE38QQR by either IV or CI from day 4 to day 10 (7 days). On day 11, organs were harvested for histologic examination.

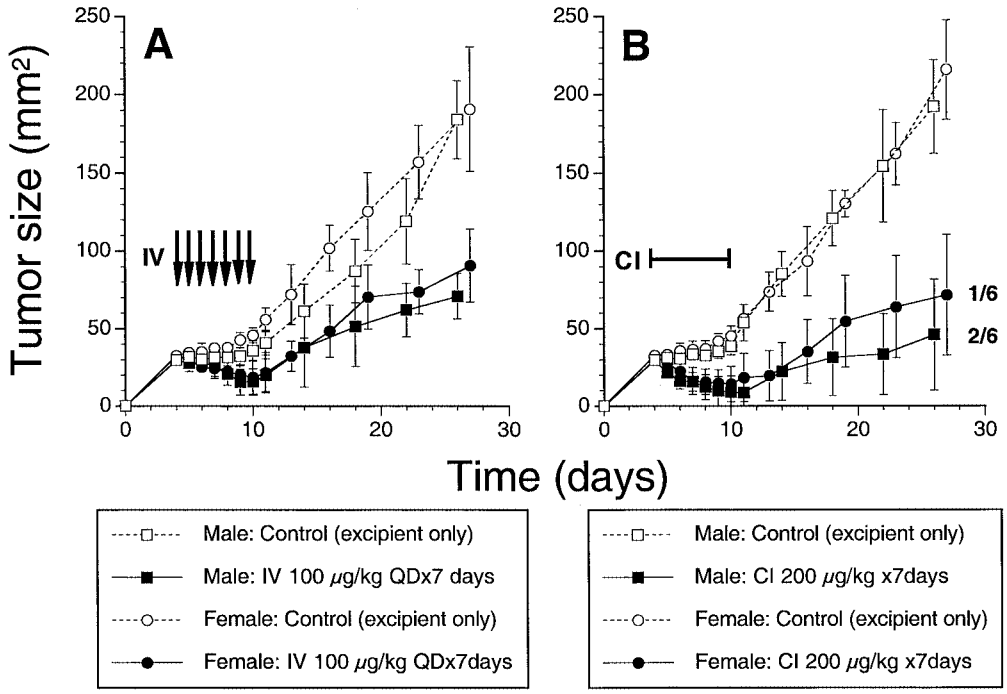

Fig. 4. Anti-tumor activity of IL-13 cytotoxin in HN12 tumor xenografted mice. Male and female mice bearing SC tumors were treated with 100 μg/kg/day IV **(A)** or 200 μg/kg/day CI **(B)** of IL13-PE38QQR from day 4 to day 10 (7 days) ($n = 6$). Bars represent the SD; CR, complete response.

smaller compared with control mice that were implanted with excipient only loaded CI pump (male, 192 mm^2; female, 216 mm^2) ($p < 0.0005$). It appears that toxicity and anti-tumor activity of IL-13 cytotoxin was not gender specific (tumor regression, male 71% versus female 67%).

Anti-tumor Activity of IL13-PE38QQR in Large SCCHN Tumor Xenografts

Finally, to assess the anti-tumor effect of IL-13 cytotoxin in large SCCHN tumor model, HN12 tumors were implanted SC in nude mice. When tumors grew to mean size of 87 ± 12 mm^2 (day 13), animals received IL-13 cytotoxin either by IV (100 μg/kg/day for 7 days) or CI (200 μg/kg/day for 7 days) routes. Excipient only injected (IV) tumors grew rapidly, reaching 199 mm^2 by the end of experiment (day 29) (Fig. 5). On the other hand, tumors treated with IL-13 cytotoxin showed growth inhibition during the treatment period by both IV and CI routes. No complete responders were generated; however, large size head and neck tumors were profoundly regressed. By day 29, mean tumor size in the IV group was 113 mm^2 and CI group was 60 mm^2, which were significantly smaller ($p < 0.0005$ in both groups) when compared to control (mean tumor size 199 mm^2). These data suggest that IL-13 cytotoxin mediates anti-tumor effects on large SCCHN tumors in a dose-dependent manner. The maximum tolerated dose by CI route was superior than maximum tolerated dose by IV route.

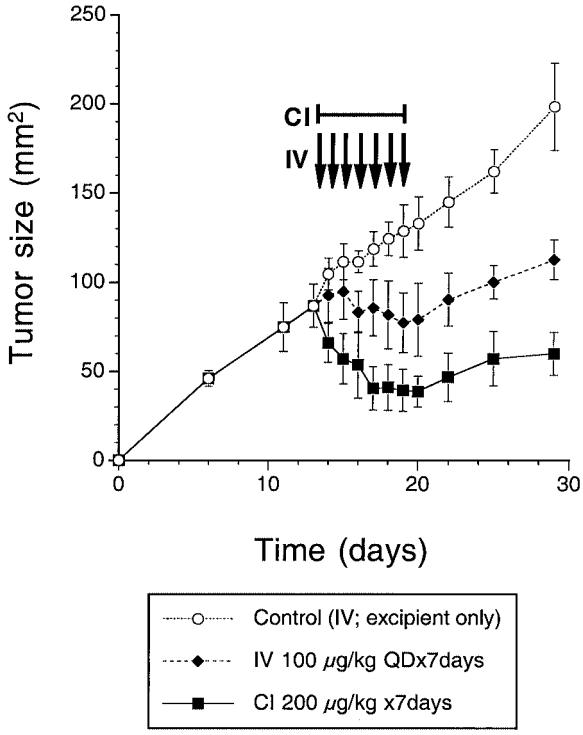

Fig. 5. Anti-tumor activity of IL-13 cytotoxin in large HN12 tumor models in mice. Male mice bearing SC large tumors (mean tumor size 87 ± 12 mm^2) were treated with 100 μg/kg/day IV or 200 μg/kg/day CI of IL13-PE38QQR from day 13 to 19 (7 days); bars, SD ($n = 7$).

Discussion

In this study, we demonstrated that systemic continuous administration decreases the toxic effects of IL-13 cytotoxin. When SC SCCHN tumor-bearing nude mice were treated with IL-13 cytotoxin by either IV or CI routes, the maximum tolerated dose by IV administration was found to be 100 μg/kg/day for 7 days, and 200 μg/kg/day for 7 days by CI route. Because CI increased the maximum tolerated dose of IL-13 cytotoxin by 2-fold, the anti-tumor therapeutic activity of IL-13 cytotoxin improved in both gender of animals.

A 50 μg/kg/day or 75 μg/kg/day dose of IL-13 cytotoxin given by IV and CI did not show any toxicities in KCCT873 tumor xenografted nude mice. Although CI treated mice showed slight superiority in anti-tumor activity, significant differences in the anti-tumor activity were not observed. HN12 xenografts treated with 150 μg/kg/day IV for 7 days had highly elevated levels of hepatic transaminases and potassium, suggesting liver toxicity. Although 300 μg/kg/day CI treated mice also showed elevated levels of these parameters, the intensity of increase was lower than in the 150 μg/kg/day IV treated mice. Histologic examinations suggested that all four vital organs—liver, kidney, lung, and spleen—were severely affected by 150 μg/kg/day IV treatment with IL-13 cytotoxin. On the other hand, organs from 300 μg/kg/day CI treatment group showed less severe toxicities compared with 150 μg/kg/day IV treated mice.

Several approaches have been tested to improve the anti-tumor activity of cytotoxins and immunotoxins and to decrease toxicity and immunogenicity of these agents. Among these approaches, site-specific PEGylation of molecule and insertion of nucleotide sequences of human immunoglobulin genes into the gene encoding mouse monoclonal antibodies have been successfully shown to prevent inadequate recruitment of host leukocytes bearing constant (Fc) region receptors (34,36,39,40). These approaches resulted into prolonged half-life of the circulating drug. In our current study, we utilized a device for CI of drug as an alternate approach to increase the availability of drug. This approach resulted in improved anti-tumor effect and decreased toxic effects. When implanted, interstitial fluid enters the CI pump via the semipermeable membrane because of the osmotic difference between the fluid and the salt solution in the pump. The fluid causes expansion of the salt layer, which compresses the flexible drug reservoir and forces solution out of the delivery portal (41). Utilizing this device, IL-13 cytotoxin can be continuously administrated systemically in body.

In summary, through CI, we successfully decreased the toxicities and increased the efficacy of IL-13 cytotoxin in tumor-bearing hosts. Because IP and IV administration of IL-13 cytotoxin has been shown to have a significant anti-tumor activity in IL-13R expressing tumors (10,16,33), its efficacy can be further enhanced by CI. We have begun Phase I/II clinical trials in patients with recurrent glioblastoma and progressive renal cell carcinoma (42). Based on our current results, we may be able to develop next generation clinical studies utilizing CI. Because IV bolus administration produces transient peak levels of drug, CI may provide constant high levels of drug exposure to tumors to enhance its systemic effectiveness.

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