Intraperitoneal radioimmunotherapy in treating peritoneal carcinomatosis of colon cancer in mice compared with systemic radioimmunotherapy

doi: 10.1111/j.1349-7006.2003.tb01498.x

Intraperitoneal radioimmunotherapy in treating peritoneal carcinomatosis of colon cancer in mice compared with systemic radioimmunotherapy

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(Received April 7, 2003/Revised May 6, 2003/Accepted May 8, 2003)

Peritoneal spread is one of major causes of mortality in colorectal cancer patients. In the current investigation, the efficacy of radioimmunotherapy (RIT) with i.p. administration of an anti-colorectal cancer IgG1, ^{131}I-A7, was compared to that with i.v. administration in BALB/c female mice bearing peritoneal nodules of LS180 human colon cancer cells, at the same toxicity level. Distribution of either i.p. or i.v. administered ^{131}I-A7 and i.p. administered irrelevant ^{131}I-HPMS-1 was assessed. Based on the results of toxicity determination at increments of 2 MBq and estimated dosimetry, an i.p. dose of 11 MBq and an i.v. dose of 9 MBq were chosen for treatment. Mice were monitored for long-term survival: untreated mice (n=11), mice undergoing i.p. RIT with ^{131}I-A7 (n=11), mice undergoing i.v. RIT with ^{131}I-A7 (n=11) and mice undergoing non-specific i.p. RIT with ^{131}I-HPMS-1 (n=5). Intraperitoneal injection of ^{131}I-A7 produced faster and greater tumor accumulation than i.v. injection: 34.2±16.5% of the injected dose per g (% ID/g) and 11.1±3.6% ID/g at 2 h, respectively (P<0.0001). Consequently, cumulative radioactivity in tumors was 1.73-fold higher with i.p. injection. ^{131}I-HPMS-1 did not show specific accumulation. Non-specific RIT with ^{131}I-HPMS-1 (mean survival, 26.0±2.5 days) did not affect the survival as compared to no treatment (26.7±1.9 days). Intravenous RIT with ^{131}I-A7 prolonged the survival of mice to 32.8±1.8 days (P<0.01). Intraperitoneal RIT with ^{131}I-A7 improved the survival more significantly and attained cure in 2 of 11 mice (P<0.05 vs. i.v. RIT). In conclusion, i.p. RIT is more beneficial in treating peritoneal carcinomatosis of colon cancer than i.v. RIT in a murine model. (Cancer Sci 2003; 94: 650–654)

Peritoneal spread is a sign of the terminal stage in colorectal cancer, being one of major causes of mortality in patients.[1–3] Intraperitoneal chemotherapy in combination with cytoreductive surgery—peritonectomy—has been examined as a therapeutic option to prolong the survival of patients,[1–3] but the majority eventually dies of progressive disease.

Several reports have indicated the effectiveness of radioimmunotherapy (RIT) employing radiolabeled monoclonal antibodies (MAb) under conditions of minimal tumor burden in cancer patients.[4–10] One of the major obstacles to RIT with i.v. injected MAb is inadequate targeting of MAb to tumors.[11] Previous studies have indicated that greater accumulation in peritoneal tumors can be achieved with i.p. injection of MAb than with systemic injection.[12–25] Furthermore, higher tumor-to-normal tissue uptake ratios are achievable with i.p. injection, resulting in a favorable dosimetry, not only as regards the radiation dose to tumors but also the toxicity profile.

Feasibility of i.p. RIT has been extensively investigated in ovarian cancer patients because of the high incidence of peritoneal dissemination of ovarian cancer[12–20]; indeed, prolonged survival with RIT has been documented. However, the effectiveness of i.p. RIT has not been fully validated for the treatment of peritoneal carcinomatosis of colorectal cancer despite reports demonstrating the dosimetric advantages of i.p. injection of MAb in peritoneal tumors.[21–25] Therefore, we aimed to compare the therapeutic efficacy of intraperitoneal RIT and i.v. RIT performed at the same toxicity level in a mouse model of human colon cancer.

Materials and Methods

Monoclonal antibodies. A7, an IgG1 murine MAb with κ-light chain, was used in this investigation.[26] It was purified from ascites of hybridoma-bearing mice by Protein A Sepharose column (Bio-Rad, Richmond, CA) chromatography. A7 MAb recognizes a 45-kD surface glycoprotein of human colonic carcinomas. The antigen is modulated after binding with A7, and the internalization of A7 occurs. A7 does not react with normal gastric mucosa, erythrocytes, peripheral lymphocytes or ileal mucosa, and shows weak reactivity with 10% of colon mucosa specimens. An additional IgG1 recognizing placental alkaline phosphatase, HPMS-1, was utilized as a class-matched irrelevant control MAb.[27] Antibodies were radiolabeled with ^{131}I by the chloramine-T method, and subsequently purified on a PD10 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). The specific activity of purified ^{131}I-A7 was 175–247 MBq/mg. Immunoreactivity of purified ^{131}I-A7 determined under antigen excess conditions with LS180 human colon cancer cell line (American Type Culture Collection, Rockville, MD) was 74–82%. The specific activity of ^{131}I-HPMS-1 was 259 MBq/mg. Antibodies were sterilized by filtration (Millex-GV, 0.22 mm; Millipore, Bedford, MA) prior to further experiments.

Peritoneal metastasis model. Animal studies were performed in compliance with the regulations of our institution. LS180 cells were grown in DMEM medium (Nissui Seiyaku, Tokyo), harvested with 0.1% trypsin, washed and subsequently suspended in PBS at 1×10^8/ml. An aliquot of the cell suspension (0.1 ml) was injected i.p. into Balb/c *nu/nu* mice (female, 20 g; NINOX Labo Supply, Inc., Ishikawa). Peritoneal nodules reach several mm in diameter by 10 days after cell inoculation.

Biodistribution of antibodies. Mice bearing peritoneal nodules were injected either i.p. or i.v. with 111 kBq (3 μCi) of ^{131}I-A7 10 days after inoculation of tumor cells. The animals were sacrificed 2 and 6 h and 1, 2 and 3 days later (n=3–5). Organs were excised and weighed; subsequently, radioactivity of the tissues was measured with a well-type γ-counter. In order to analyze the relationship between metastasis size and ^{131}I-MAb ac-

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cumulation, nodules of various sizes were selected at random. Nodules were individually weighed and counted for radioactivity. The biodistribution of i.p. injected ^{131}I-HPMS-1 was similarly observed 1 and 2 days after injection as a negative control (n=3), and the results were analyzed by means of the Mann-Whitney test. The level of significance was set at 5%.

Determination of toxicity. Toxicity was determined for i.p. and i.v. administration of ^{131}I-A7 by assessing thrombocytopenia and body weight loss after ^{131}I-A7 administration at increments of 2 MBq. A blood sample (5 μl) was obtained from a tail vein of each mouse. Samples within a single group (n=3) were pooled and diluted 1:100 in 1% ammonium oxalate for platelet counts.

For the dosimetric assessment, cumulative radioactivity after ^{131}I-A7 administration in 1 g of tissue (MBq×h/g) was obtained by the trapezoid integration method using the biodistribution data.

Radioimmunotherapy. The therapeutic experiment consisted of four groups: non-treated mice (n=11), mice undergoing specific RIT with i.p. injection of ^{131}I-A7 (n=11), mice undergoing specific RIT with i.v. injection of ^{131}I-A7 (n=11), and mice undergoing non-specific RIT with i.p. injection of an irrelevant ^{131}I-HPMS-1 (n=5). Administration doses were chosen based on the results of toxicity determination. The dose of ^{131}I-HPMS-1 was set at the same level as that of ^{131}I-A7. MAbs were administered 10 days after inoculation of tumor cells. Animals were monitored for survival. Results were analyzed by the Kaplan-Meier method with the log rank test. The level of significance was set at 5%.

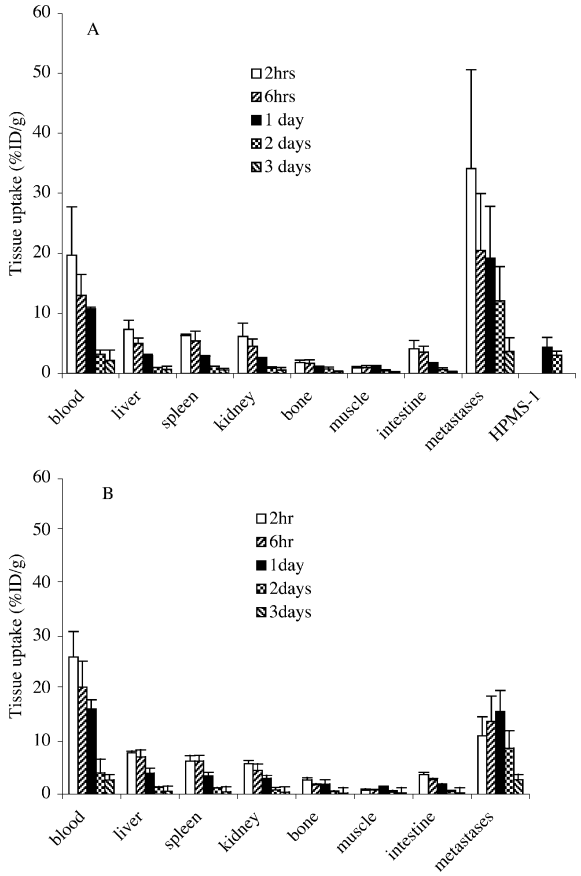

Fig. 1. Biodistribution of i.p. injected ^{131}I-A7 (A) and i.v. injected ^{131}I-A7 (B) in mice bearing peritoneal metastases (n=3–5). Accumulation of an irrelevant ^{131}I-HPMS-1 in metastases is also shown (A) (n=3). Values of tumor accumulation are means±SD of all samples obtained in each group. In general, 10–20 metastatic nodules of various sizes ranging 0.1–400 mg were measured for antibody accumulation in each mouse. No significant differences were observed in the distribution of lesional sizes among observation groups.

Results

Intraperitoneal injection of ^{131}I-A7 resulted in faster and greater accumulation than i.v. injection: 34.2±16.5% ID/g and 11.1±3.6% ID/g at 2 h after injection, respectively (P<0.0001) (Fig. 1, A and B). On the other hand, the i.v. route yielded peak activity in tumors 1 day after injection (Fig. 1B). Radioactivity level in tumors was similar in the two groups at late time points. Accumulation in tumors increased with decreasing size in both administration routes (Fig. 2). An irrelevant MAb,

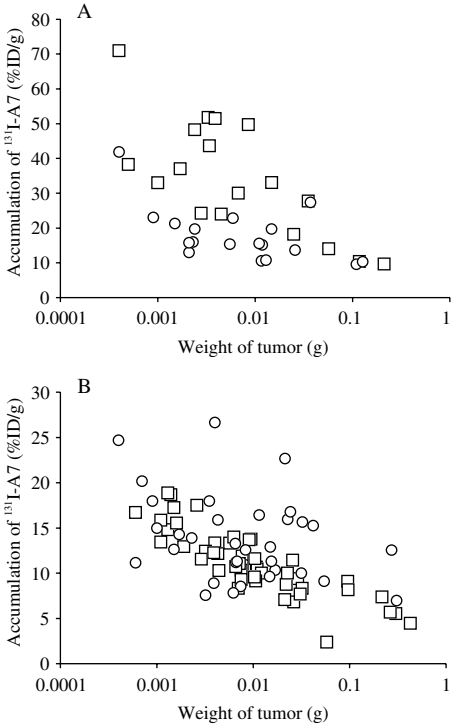

Fig. 2. Size-effect of peritoneal metastatic nodules on accumulation of ^{131}I-A7 at 2 h (□) and 6 h (○) after i.p. (A) or i.v. (B) injection.

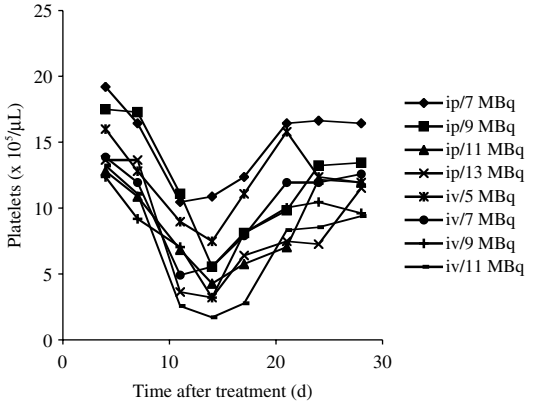

Fig. 3. Change of platelet count induced by ^{131}I-A7 administered either i.p. or i.v.

HPMS-1, did not specifically accumulate in tumors (Fig. 1A).

Toxicity of ^{131}I-A7 administration was dose-dependent (Fig. 3 and Fig. 4). Because the body weight of mice receiving an i.v. dose of 11 MBq was greatly decreased and recovery was poor, the second highest dose, 9 MBq, was chosen for the i.v. dose in therapeutic assessment. Based on the degrees of platelet depression and body weight loss, an i.p. dose of 11 MBq was determined to be equitoxic. According to these results, cumulative radioactivity values in tissues were calculated for the administration of i.v. 9 MBq and i.p. 11 MBq (Table 1). Cumulative radioactivity in normal tissues was nearly identical after i.v. 9 MBq and i.p. 11 MBq, validating the results of toxicity determination. At these administration doses, i.p. injection of ^{131}I-A7 produced 1.73-fold higher cumulative radioactivity in peritoneal tumors than i.v. administration, and therapeutic ratios relative to normal organs were significantly higher with i.p. injection.

All mice with peritoneal metastases died by 33 days after cell inoculation if no treatment was conducted (Fig. 5). Mean survival of non-treated mice was 26.7±1.9 days. Non-specific RIT with an irrelevant ^{131}I-HPMS-1 (mean survival, 26.0±2.5 days), did not affect the survival as compared to no treatment. Intravenous RIT with ^{131}I-A7 prolonged the survival of animals to 32.8±1.8 days ($P<0.01$). Intraperitoneal RIT with ^{131}I-A7 improved the survival more significantly ($P<0.05$). Moreover, i.p. RIT attained cure in 2 of 11 mice.

Discussion

RIT currently utilizes β emitters to treat cancer lesions. Clinical RIT in patients with bulky solid tumors has shown only limited success.[11] On the other hand, recent investigations have indicated the role of RIT in an adjuvant setting where small residual tumors are targeted by radiolabeled MAb,[4–10] despite the limitations of β emitters suggested by mathematical model analyses.[28] A factor influencing the effectiveness of RIT in small tumors is the size-dependency of MAb accumulation in tumors.[29, 30] In the current investigation, we found that this situation is also true in targeting i.p. metastases of 0.1–400 mg.

One of the major reasons for therapeutic failure of clinical RIT in solid tumors is an insufficient radiation dose to tumors.[11] To overcome this limitation, locoregional delivery of labeled MAb has been proposed, especially in ovarian cancer patients because of the high incidence of peritoneal dissemination of ovarian cancer.[12–20] In colorectal cancer, the dosimetric advantage of i.p. administration has been similarly acknowledged[21–25]; however, the effectiveness of i.p. RIT relative to i.v. RIT has not been validated so far. The current investigation confirmed better therapeutic effects as well as more favorable dosimetry of i.p. RIT as compared to i.v. RIT in a peritoneal metastasis model.

Because of Michaelis-Menten kinetics in antibody-antigen interactions,[31] the higher MAb concentration in the peritoneal cavity achieved by i.p. administration than i.v. administration is beneficial. Slow absorption of large molecules such as MAbs from the peritoneal cavity may maintain high concentration.[32] These factors cause rapid and intense localization of MAb in peritoneal tumors, resulting in considerably higher therapeutic ratios as compared to those with i.v. administration. However,

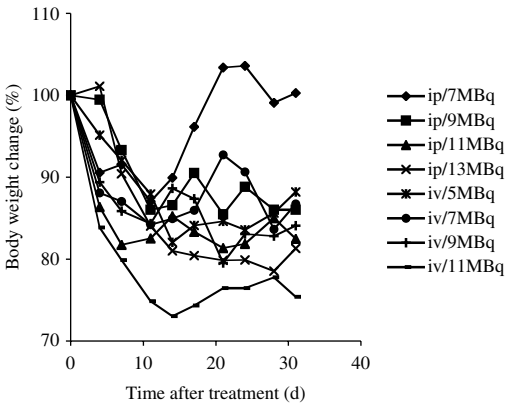

Fig. 4. Body weight loss induced by ^{131}I-A7 administered either i.p. or i.v.

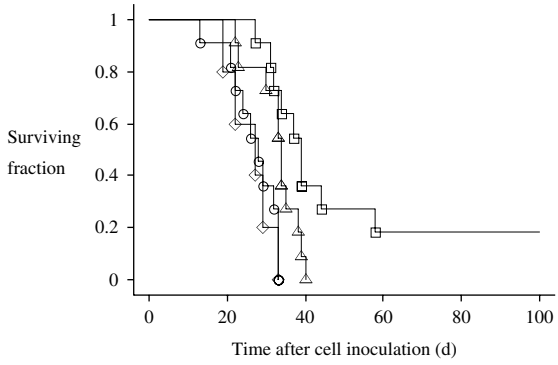

Fig. 5. Survival of mice bearing peritoneal metastases. Radioimmunotherapy (RIT) was performed 10 days after cell inoculation. ○, non-treated control; ◇, non-specific RIT with an irrelevant ^{131}I-HPMS-1 i.p. injected; △, i.v. RIT with ^{131}I-A7; □, i.p. RIT with ^{131}I-A7. Doses of i.p. RIT and i.v. RIT were 11 MBq and 9 MBq, respectively.

Table 1. Cumulative radioactivity after i.p. or i.v. administration of ^{131}I-A7

	Cumulative radioactivity (MBq×h/g)		Metastasis-to-normal tissue ratio		
	i.p.	i.v.	i.p.	i.v.	i.p./i.v.
Blood	61.3	70.4	1.75	0.88	1.98
Liver	17.6	18.8	6.09	3.29	1.85
Spleen	18.1	16.6	5.93	3.73	1.59
Kidney	15.6	13.2	6.86	4.70	1.46
Bone	7.2	7.0	14.80	8.83	1.68
Muscle	5.3	4.3	20.17	14.31	1.41
Intestine	11.1	8.3	9.62	7.45	1.29
Metastasis	107.0	62.0	—	—	—

i.p., intraperitoneal administration (11 MBq); i.v., intravenous administration (9 MBq); i.p./i.v., ratio of metastasis-to-normal tissue ratio of i.p. administered ^{131}I-A7 to that of i.v. administered ^{131}I-A7.

this advantage was less prominent at later time points, which suggests that RIT employing radionuclides with physical half life shorter than ^{131}I would deliver radiation to tumors more effectively.

Liver metastasis occurs more often than peritoneal spread in colon cancer patients, and both types of metastases may simultaneously exist. We have previously demonstrated the effectiveness of RIT in a liver metastasis model of colon cancer in which radio-labeled A7 was administered systemically.[33–35] Intraperitoneal MAb administration reportedly produces a similar degree of MAb accumulation in liver metastases as compared to i.v. injection,[36] so that i.p. RIT may work in treating liver metastases as well as peritoneal lesions.

Previous studies demonstrated the improvement of therapeutic outcomes with combination treatment of RIT and chemotherapy in comparison with monotherapy.[37, 38] For instance, specificity of 5-FU against cells in the S-phase of the cell cycle suggests that 5-FU may act selectively against less radiosensitive cells.[39] Furthermore, chemotherapeutic drugs may act as a radiosensitizer so that the interaction with RIT may not be a simple additive effect, but rather a synergistic effect.[39] This combination therapy seems worth evaluating in a peritoneal metastasis model.

In conclusion, i.p. administration of ^{131}I-A7 affords an improvement in terms of absorbed radiation dose to colon cancer metastases within the peritoneal cavity in a murine model, leading to longer survival in mice treated via the i.p. route in comparison with the i.v. route.

We thank former Professor Toshio Takahashi and Dr. Toshiharu Yamaguchi, First Department of Surgery, Kyoto Prefectural University of Medicine, for providing A7 MAb. This study was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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