Comparison of Multiple Bolus and Continuous Injections of $^{131}$I-labeled CC49 for Therapy in a Colon Cancer Xenograft Model $^1$

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Abstract

One of the problems in achieving cures with radioimmunotherapy is that hematological toxicity limits the quantity of radiolabeled monoclonal antibody (MAb) that can be administered. The MAb CC49 binds with high affinity to the TAG-72 antigen expressed in many human adenocarcinomas. We investigated tumor growth inhibition, survival, and tumor and bone marrow dosimetry after multiple bolus injections or continuous infusion of $^{131}$I-labeled CC49 MAb in a human colon cancer xenograft model to determine which method of administration results in the highest therapeutic ratio. Groups of athymic nude mice bearing established s.c. LS174T human colon cancer xenografts received three i.p. bolus injections (3X) or $^{131}$I-labeled CC49 (3X, days 0, 3, and 7) or were implanted i.p. with mini-osmotic pumps delivering $^{131}$I-labeled CC49 over 7 days. The total radionuclide doses administered were broken down into low-dose ($<450$ μCi), medium-dose (450–800 μCi), and high-dose (>800 μCi) groups. At the medium-dose level, the bolus-therapy animals did not have a significantly longer survival time but did have a significantly longer time-to-tumor doubling than the pump-therapy animals. The median survival for medium-dose bolus and pump therapy was 157 and 105 days, respectively, and the median time-to-tumor doubling was at least 114 and 77 days, respectively. At the low-dose level, the bolus-therapy animals had a significantly longer survival time but not a significantly longer time-to-tumor doubling than the pump-therapy animals. The median survival for low-dose bolus and pump therapy was 95.5 and 59 days, respectively, and the median time-to-tumor doubling was 73 and 38 days, respectively. The high-bolus dose was toxic. A comparison of the overall survival rate of pump therapy versus bolus therapy, excluding high-dose, resulted in the bolus-therapy animals having a longer survival time and a longer time-to-tumor doubling than the pump-therapy animals.

Serial section autoradiography was used to reconstruct tumor activity density distributions over time. Average dose values calculated from total uptake data for 900 μCi administered activity yielded 158 Gy (3X) and 141 Gy (pump). Average three-dimensional doses using the radial histograms to calculate the absorbed fractions were 139 Gy and 123 Gy, respectively. This calculation includes energy loss external to the tumor. With cell proliferation parameters set to single fraction $^{60}$Co recurrence results, the effective dose ($D_{eff}$) for local control was 11 Gy and 9 Gy, respectively. Three bolus injections resulted in a more uniform dose rate over a longer period, resulting in a calculated 19% improvement in $D_{eff}$ compared with pump administration. Dose to bone marrow was calculated assuming an activity concentration in bone marrow of 0.24 times the concentration in blood and an absorbed fraction of 0.63. For the 900-μCi $^{131}$I-labeled CC49 injected activity, pump administration resulted in an 80% higher calculated $D_{eff}$ to bone marrow compared with 3X bolus injection.

These results demonstrate that 3X bolus injections were clearly superior to pump administration in terms of survival, tumor growth inhibition, tumor absorbed dose, and bone marrow dose.

Introduction

Radiolabeled MAbs$^3$ have been investigated in animal models for RIT of human colon cancer xenografts ($1, 2$) in an attempt to develop promising approaches to improving therapy success in patients. Effective RIT requires the selective delivery of these reagents to tumor cells at a high enough concentration to kill a large fraction of the tumor cells. Strategies to increase the uptake and dose of radiolabeled MAbs in tumors while at the same time minimizing their uptake and dose in normal tissues would improve the time-dependent tumor/normal tissue dose ratios so that higher and more frequent doses of radionuclide could be used for RIT to achieve a maximum therapeutic ratio ($3$).

The use of high affinity MAbs is one strategy that has been evaluated for increasing the uptake and retention of radiolabeled MAbs in tumor xenografts and increasing their therapeutic efficacy ($4$). Muraro et al. ($5$) produced MAbs with different...
affinities for the TAG-72 antigen expressed in many human adenocarcinomas (6–10). A direct correlation was found between the affinity of these MAbs to purified TAG-72 and their localization and therapeutic efficacy against LS174T human colon cancer xenografts (4, 11). On the basis of these studies, we selected the high affinity anti-TAG-72 murine CC49 MAb for the present studies.

One of the difficulties in achieving cures with RIT is that hematological toxicity limits the quantity of radiolabeled antibody that can be administered. The use of dose fractionation involving multiple injections of radiolabeled MAbs is a strategy for producing more prolonged tumor growth inhibition, reducing hematological toxicity, and allowing for higher total-doses of radionuclide to be administered than in a single administration (2, 12–15). Continuous infusion is an extrapolation to an infinite number of injections and may be useful for improving tumor uptake as a result of higher vascular permeability or a more homogeneous intratumor distribution of radiolabeled MAbs while minimizing bone marrow toxicity. In a previous study comparing the biodistribution of 131I-labeled CC49 in athymic nude mice bearing LS174T s.c. xenograft after bolus injection or continuous infusion, the highest tumor concentrations of 131I-labeled CC49 MAb were achieved after i.p. bolus injection (16). The highest tumor:blood ratios were achieved after a single i.p. bolus injection, because of the higher tumor uptake and more rapid blood clearance.

The purpose of this study was to investigate the comparative therapeutic efficacy of 131I-labeled CC49 MAb in athymic nude mice bearing LS174T colon cancer s.c. xenografts after multiple bolus injections or continuous infusion to determine which method of administration produces the highest tumor growth inhibition and survival and to estimate tumor and bone marrow dosimetry.

Materials and Methods

MAb and Cell Lines. The purified mouse MAb CC49, of the IgG1k subclass, reactive with the TAG-72 antigen expressed on human adenocarcinomas (5) was kindly provided by Dr. Jeffrey Schlom (National Cancer Institute, Bethesda, MD). The LS174T human colon adenocarcinoma cells were obtained from the American Type Culture Collection (Rockville, MD) and were grown as a monolayer in T-75 flasks (Corning, Corning, NY) according to previously described procedures (13). Serial passaging and harvesting of cells during the log phase of growth for injection into athymic nude mice was accomplished with trypsin-versene (0.05% trypsin, 0.02% versene, Whittaker M.A. Bioproducts, Walkersville, MD).

Radiolabeling of MAb. Purified MAb CC49 was labeled with 131I (New England Nuclear, N. Billerica, MA) as described previously and stored in 5% human serum albumin (17).

Immunoreactivity Determination. The immunoreactivity of radiolabeled antibody preparations was measured using BSM (Sigma Chemical, St. Louis, MO) coated beads. The BSM was immobilized onto a solid support (6.2-mm polystyrene beads; Precision Plastic Ball, Franklin Park, IL) at a concentration of 10 µg BSM per bead. The radiolabeled MAbs were prepared at 10 ng/ml in 1% BSA in PBS and added in duplicate (100 µl) to a 12 × 75-mm glass test tube containing a single BSM bead in the absence and presence of increasing concentrations of unlabeled MAb. The tubes were counted in a gamma scintillation counter and incubated for 1 h at room temperature over a laboratory oscillator. The BSM beads were washed with 4 ml of PBS, and the radioactivity remaining in each tube was measured. The total percent of radioactivity bound to the BSM beads was calculated and the inverse of % binding was plotted versus the inverse of the concentration of unlabeled MAb. The inverse of the y intercept was determined as percentage immunoreactive fraction.

Mice. Athymic nude female nu/nu mice with a BALB/c background, 4–5 weeks old, were obtained from the National Cancer Institute Frederick Research Laboratory (Frederick, MD). Mice were kept under sterile conditions in a laminar flow room in cages with filter bonnets and were fed sterilized mouse diet and sterilized tap water. The procedures used to minimize discomfort, distress, and pain to the animals were in accordance with the Animal Resource Program at the University of Alabama at Birmingham, accredited by the American Association for Accreditation of Laboratory Animal Care.

Establishing Colon Tumors in Athymic Nude Mice. LS174T tumor cells were harvested and suspended in sterile PBS at a concentration of 5 × 107 viable cells/ml. Cell viability was determined by trypan blue dye exclusion. Cells (1 × 107 viable) in sterile PBS were injected s.c. into the flank of nude mice. When the tumors were approximately 5–10 mm in diameter (7–9 days postinjection), animals received 131I-labeled CC49 MAb as described below. The tumor sizes were comparable among groups.

Therapy Studies with 131I-labeled CC49 Antibody. For the therapy analysis, groups of mice bearing established tumors of 5–10 mm in diameter received three i.p. bolus injections of 131I-labeled CC49 (5X) in 0.2 ml of saline on days 0, 3, and 7. The triple bolus injections were prepared in two radio-labelings. The first radiolabeling was used for the day 0 and 3 injections. Other groups of mice were implanted i.p. with Alzet mini-osmotic pumps (Model 2001, Alza Corp., Palo Alto, CA) prepared to deliver 131I-labeled CC49 in a reservoir volume of 200 µl with a mean pumping rate of 1.0 (±0.15) µl/h over a 7-day period. The mini-osmotic pumps were filled aseptically according to the manufacturer’s instructions with 131I-labeled CC49 diluted in PBS containing 5% human serum albumin. The pumps were implanted i.p. as described previously (16). The pumps were removed at day 7 after implantation. All of the pumps were weighed and counted in a dose-calibrator (Capintec Model CRC-15R, Ramsey, NJ) before implantation and after removal to measure the dose of 131I-labeled CC49 administered, taking into account radionuclide physical decay. Some of the animals died early because of the pump installation or removal and others because of radionuclide toxicity. Because varying doses of both pump and bolus therapy were tested, overall survival and tumor growth measures would be inappropriate. Therefore, the doses were broken down into low, medium, and high doses. Low doses correspond to any dose at or below 450 µCi; medium doses are those that are greater than 450 µCi but less than 800 µCi; and high doses are those greater than 800 µCi. Therefore, separate survival analysis comparisons were done comparing pump versus bolus therapy for low- and
medium-dose levels (no pump therapy animals received a high dose).

**Tumor and Bone Marrow Dosimetry.** Comparison three-dimensional tumor dosimetry was performed for athymic nude mice bearing LS174T s.c. xenografts given three bolus i.p. injections (3X, days 0, 3, and 7) or continuous i.p. infusion via mini-osmotic pumps over 7 days of $^{131}$I-labeled CC49 MAb. Serial section autoradiography was used to reconstruct tumor activity density distributions for days 3, 4, 7, 8, and 11 (3X), or days 4, 7, 10, and 13 (pump) as described previously (18). Uptake in blood and tumor was measured up to 11 days (3X) or 16 days (pump) postinjection. Tumor dose values were calculated by two methods. The first method used total activity uptake data, summed using the trapezoidal rule, and the MIRD uniform isotropic model, assuming no energy loss external to the tumor. The second, more accurate method used three-dimensional reconstructions based on serial section autoradiography (~15 sections per tumor) as described previously (18–21). At least three tumors per time point were reconstructed. The reconstructed activity density distribution for each tumor was converted to a dose-rate distribution by convolving the activity density distribution with a dose voxel kernel derived from a dose point kernel. The dose-rate calculation used the actual geometry of each tumor. For the purpose of tumor dose model construction, the dose distribution for each tumor was mapped to radial histograms, retaining the range of dose rates from the original dose-rate distribution. The radial histograms represented radial shells from the tumor. Each voxel assigned its average value of each histogram was used to illustrate radial dose-rate histograms for each time point. The averaged radial dose-rate histograms $\{R(r, AV, t)\}$, a function of the radius $r$, voxel $AV$, and time $t$ represented a time-dependent three-dimensional dose-rate distribution. The volume elements represented in the radial histograms were registered between time points assuming the approximation of maximum heterogeneity. Cumulative dose was calculated using

$$D(r, AV, t) = \sum_{r'=0}^{r} R(r, AV, t')$$  \hspace{1cm} (A)

The average tumor dose was calculated by averaging over voxels for infinite time. The linear quadratic model with fractional cell survival ($S$) was used to define a $D_{eff}$.

$$D_{eff} = \frac{1}{\ln(S)}$$  \hspace{1cm} (B)

The tumor cell proliferation constant was set to match single-fraction $^{60}$Co recurrence results ($\gamma = \ln (2)/t_d$ where $t_d = 3.4$ days is the cell doubling time). The $D_{eff}$ calculation used the time-dependent radial dose histograms, the radial dependence of the necrotic and hypoxic fractions, and the proliferation rate to determine the fractional cell survival.

$$S(t) = \frac{1}{V} \sum \exp[-D(r, AV, t)RE(r, AV, t) + \gamma t]$$  \hspace{1cm} (C)

where $RE(r, AV, t)$ is the relative effectiveness describing the dose-rate response according to the linear quadratic model (18) and $V = \sum \Delta V$. The $D_{eff}$ was calculated using the minimum $S(t)$.

Bone marrow dose was calculated using the integrated area under the blood uptake curve, assuming a bone marrow:blood concentration factor of 0.24 (22) and an absorbed fraction in bone marrow of 0.63 (23). The linear cell loss factor was chosen as typical of published values in the mouse, $\alpha_{BM} = 1.1$ Gy$^{-1}$ (24). The proliferation constant was chosen based on the doubling time for colony forming units, $\gamma_{BM} = \ln (2)/t_d$ where $t_d = 3.2$ days (25). The $D_{eff}$ was calculated using the minimum $\gamma_{BM}(t)$, where

$$\gamma_{BM}(t) = \exp[-\alpha_{BM}D(t) + \gamma_{BM}]$$  \hspace{1cm} (D)

**Statistical Analysis.** The log-rank test was used to compare survival distributions and time-to-tumor doubling between various groups of animals. These comparisons—made for only those animals that survived at least 10 days—resulted in a comparison of tumor-related survival time and tumor growth.

**Results**

**Radiolabeling and Immunoreactivity.** The $^{131}$I-labeled CC49 preparations had specific activities of 6–10 nCi/mg. Gel filtration high-pressure liquid chromatography of the $^{131}$I-labeled CC49 MAb preparations indicated that the products were homogeneous, and that >95% of the radiolabel was protein-associated immediately after radiolabeling. However, the percentage of free $^{131}$I increased to 11% when stored at 37°C for 7 days.

$^{131}$I-labeled CC49 retained its tumor cell specificity and immunoreactivity after radiolabeling, with 83% of the antibody binding to mucin-coated beads. However, the immunoreactivity decreased to 67, 53, 40, and 31% when the $^{131}$I-labeled CC49 was stored at 37°C for 1, 2, 5, and 7 days, respectively.

**Therapy Studies with $^{131}$I-labeled CC49.** At the medium dose level, the bolus-therapy animals did not have a significantly longer survival time but did have a significantly longer time-to-tumor doubling than the pump-therapy animals ($P = 0.0542$ and $P = 0.0001$, respectively; Fig. 1). The median survival for medium-dose bolus and pump therapy was 157 and 105 days, respectively, and the median time-to-tumor doubling for medium-dose bolus and pump therapy was at least 114 and 77 days, respectively. At the low-dose level, the bolus-therapy animals had a significantly longer survival time but not time-to-tumor doubling than the pump-therapy animals ($P = 0.0133$ and $P = 0.1264$, respectively). The median survival for low-dose bolus and pump therapy was 95.5 and 59 days, respectively, and the median time-to-tumor doubling for low-dose bolus and pump therapy was 73 and 38 days, respectively.

For those animals that received bolus therapy, there was significant difference in overall survival and time-to-tumor doubling ($P = 0.0001$ and $P = 0.0017$, respectively) among the three dose groups (Fig. 2). The median survival time for the low-, medium-, and high-dose bolus therapy was 95.5, 157.0, and 18.0 days, respectively, and the median time-to-tumor doubling for the low-, medium-, and high-dose bolus therapy was 73, at least 114, and at least 4 days, respectively. It is evident that the high dose was extremely toxic, but the low and espe-
3156s Therapy with 131I-labeled CC49

Therapy with 131I-labeled CC49

0.8 (Dc-O "E 0.4

0.2 N=26

N=10

50 75 100 125 150 175 200

Days

1.0 -B N = 17

-~ 0.8 N=47 N=10

0.6

0.4

25 50 75 100 125 150 175 200

Days

Fig. 1 Survival analysis (A) and time-to-tumor doubling (B) of athymic nude mice bearing LS174T human colon carcinoma xenografts versus time comparing pump versus bolus for low-dose (less than 450 txCi) and medium-dose (450–800 µCi) injections of 131I-labeled CC49. ▲, medium bolus dose; ○, medium pump dose; ●, low bolus dose; ■, low pump dose.

Fig. 2 Survival analysis (A) and time-to-tumor doubling (B) of athymic nude mice bearing LS174T human colon carcinoma xenografts versus time comparing low-dose (less than 450 txCi), medium-dose (450–800 µCi), or high-dose (greater than 800 µCi) bolus injections of 131I-labeled CC49. ●, medium dose; ○, low dose; ▲, high dose.

especially the medium dose produced long survival times and time-to-tumor doubling.

A comparison of the overall survival rate of pump versus bolus therapy, excluding high dose, results in the bolus-therapy animals having a longer survival and time-to-tumor doubling than the pump-therapy animals (P = 0.0103 and P = 0.0001, respectively). The median survival time for bolus- and pump-therapy animals was 140 and 74 days, respectively, and the median time-to-tumor doubling for bolus versus pump therapy was at least 123 and 49 days, respectively.

Tumor and Bone Marrow Dosimetry. Comparison three-dimensional tumor dosimetry was performed for mice given 3X or continuous i.p. infusion of 300 µCi 131I-labeled CC49 MAb. The 3X and pump autoradiographs of tumors at early time points displayed a more uniform-appearing activity distribution than the later distributions, which showed surface uptake (18). The three-dimensional average radial dose-rate distributions were significantly different for the 3X and pump studies for day 7 (Fig. 3), in which the 3X uptake was more concentrated at the surface. For days 10 and 11 postinjection, the radial distributions were similar (Fig. 4). The 3X bolus technique compared with the pump technique was able to sustain increased surface uptake over a longer period of time.

The calculated average and D_eff to tumor are shown in Table 1. The calculated values are normalized to 3 x 100 µCi, and 300 µCi, 600 µCi, and 900 µCi for the pump injections. Doses calculated from activity-uptake data using the MIRD uniform isotropic model (no energy loss external to the tumor) yielded 158 Gy (3X) and 141 Gy (pump) for the 3 x 300-µCi and 3 x 900-µCi injections, respectively. The first row of Table 1 lists average tumor doses calculated using absorbed fractions derived from the radial histograms. This calculation includes energy loss external to the tumor. Average three-dimensional doses for 3 x 300-µCi (3X injections) and 900-µCi (pump injections) administered activities were 139 Gy and 123 Gy, respectively. The nonuniformity of dose deposition impacts the treatment outcome because cell loss is an exponential function of dose. Tumor cell proliferation also impacts outcome for variable dose-rate treatments. The linear quadratic model with fractional cell survival and cell proliferation was used to define a D_eff. With cell proliferation parameters set to match single-fraction 60Co recurrence results, the D_eff was 11.1 Gy and 9.3 Gy, respectively. Three bolus injections produced a higher surface dose rate over a longer period and a more rapid initial dose delivery, resulting in a 19% improvement in the calculated D_eff compared with pump infusion.
Fig. 3 Radial dose-rate curves for three fractions (3X) and continuous infusion (pump) for early postinjection time. The radial curves were generated from the average values of each of the radial histograms and are normalized to unit average dose rate assuming no energy loss external to tumor. The tumor surface is at radius = 1.0. Data were analyzed for 12 tumors (pump) and 4 tumors (bolus), respectively.

Fig. 4 Radial dose-rate curves for 3X and pump injections for late postinjection times. The radial curves were generated from the average values of each of the radial histograms and are normalized to unit average dose rate assuming no energy loss external to tumor. The tumor surface is at radius = 1.0. Data were analyzed for 3 tumors (pump) and 13 tumors (bolus), respectively.

Dose-to-bone marrow was calculated assuming an activity concentration in bone marrow of 0.24 times the concentration in blood and an absorbed fraction of 0.63. Bone marrow doses and $D_{\text{eff}}$s are shown in Table 2. This proliferation model would yield the same result as long as the ratio of $\gamma_{\text{blood}}/\gamma_{\text{BM}}$ was constant. The average doses include the loss of energy external to the marrow and the contribution from the whole body. Because the $D_{\text{eff}}$ calculation varies non-linearly with injected activity, the $D_{\text{eff}}$ values are not proportional to injected activity. For 900-$\mu$Ci injected activity, pump administration resulted in an 80% higher $D_{\text{eff}}$-to-bone marrow than 3X bolus injection. The ratio of $D_{\text{eff}}$s was greater at lower injected activities.

Discussion

High-dose RIT for patients with gastrointestinal tumors has resulted in fewer objective responses than achieved in patients with lymphomas (26). The poor success in treating gastrointestinal cancers and other solid tumors with RIT is likely related to their lower radiosensitivity and more heterogeneous intratumor distribution of radiolabeled MAbs as compared with lymphomas (21, 27). Methods to increase the uptake and radiation absorbed dose of radiolabeled MAbs in solid tumors while reducing the uptake and absorbed dose in normal tissues are needed so that a sufficient radiation-absorbed-dose will be delivered for the curative treatment of such radioresistant tumors. This was the basis for investigating the use of mini-osmotic pumps for continuous-infusion versus multiple-bolus injections.

The use of dose fractionation involving multiple bolus injections of radiolabeled MAbs is a strategy for reducing hematological toxicity as a result of the repair of radiation damage.
in bone marrow progenitor cells between fractions. Fractionated dose administration reduces hematological toxicity and allows for higher total doses of radionuclide to be administered (12, 15). The use of multiple bolus injections or continuous infusion of radiolabeled MAbs may result in higher tumor-uptake and greater therapeutic efficacy as a result of higher vascular permeability (as has been shown after external beam irradiation) or in more homogeneous intratumor distribution of the radiolabeled MAbs as a result of the uptake of each fraction at the tumor surface, where surviving cells are proliferating (21, 28–30).

Experiments with fractionated- and continuous-injection schemes have shown that tumor-uptake curves for multiple fractions and continuous injections are not simple linear superpositions of single-injection uptake curves (18). Later fractions typically have less uptake as a percentage of injected antibody dose than the initial fraction. Possible contributors to this effect are: (a) vessel scarring or other effects restricting the movement of antibody into the intercellular space (31); and (b) the reduction in the mass of viable tissue due to cell death together with a mostly peripheral cell population in a continuous process of expansion and angiogenesis (32). The latter effect is attributed to the positive result of the radiation therapy. Therefore, some reduction of uptake would be expected. An understanding of the uptake dependence is essential for the prediction of optimum fractionation frequency. Whether the reduction in uptake is due to vessel scarring or to the reduction of viable tumor volume, there seems to be a dose-response relationship. There is also a time relationship, manifesting itself as a lag time between dose delivery and reduction of uptake (31).

These results demonstrate that multiple-bolus injection was clearly superior to pump administration in terms of survival, tumor-growth inhibition, tumor-absorbed-dose, and bone-marrow dose. This was partially due to radiolabeled antibody degradation over the 7-day pumping period at 37°C. The delay in tumor uptake and the ever-present antibody in the blood for the pumping experiments also contributed. Within the bolus-injection groups, the medium-dose group had a significantly better survival than the high- and low-dose groups. Although the tumor growth could not be distinguished between the high and medium doses, the low-survival rate in the high-dose group would suggest that the superior method of administration was bolus therapy at medium-dose levels.

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References


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